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Differing Routes to Stem Cell Research: Germany and Italy

edited by

Renato G. Mazzolini / Hans-Jörg Rheinberger

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DIFFERING

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Stem Cell Research in Germany with Specific Regard to Human Embryonic Stem Cells

by *Anna M. Wobus** and *Peter Löser***

I. HISTORICAL REFLECTIONS

Research on pluripotent stem cells dates back to the middle of the last century, when Leroy Stevens discovered the relationship between early embryos and the formation of teratocarcinomas, these being transplantable tumours containing embryonic carcinoma (EC) cells – the stem cells of malignant teratocarcinomas¹. *In vitro* cultivation and characterization of EC cells revealed self-renewal and differentiation as properties characteristic of stem cells².

Research on EC cells in those early days – summarized at the Cold Spring Harbor Conference on «Teratocarcinoma Stem Cells» in September 1982 – marked the advent of pluripotent stem cell research³. Although it became evident that EC cells developed chromosomal aberrations resulting in a lack of pluripotency after integration into mouse embryos,

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** Robert Koch Institute, Berlin, Germany.

We are grateful to Dr. Tobias Grimm (DFG) and Dr. Andreas Künne (BMBF) for helpful information. We also wish to thank Anke Guhr and Sabine Kobold for support in data acquisition.

¹ L.C. STEVENS, *The Development of Transplantable Teratocarcinomas from Intratesticular Grafts of Pre- and Postimplantation Mouse Embryos*, in «Developmental Biology», 21, 1970, pp. 364-382.

² See A.M. WOBUS - K.R. BOHELER, *Embryonic Stem Cells: Prospects for Developmental Biology and Cell Therapy*, in «Physiological Reviews», 85, 2005, pp. 635-678; A.M. WOBUS, *The Janus Face of Pluripotent Stem Cells – Connection between Pluripotency and Tumourigenicity*, in «BioEssays», 32, 2010, pp. 993-1002.

³ L.M. SILVER - G.R. MARTIN - S. STRICKLAND (eds), *Teratocarcinoma stem cells* (Cold Spring Harbor Conferences on Cell Proliferation, 10), Cold Spring Harbor NY 1983.

this research provided the basis for later studies involving embryonic stem cells (ESCs): In 1981, based on their work with *in vitro* cultivated mouse EC cells, Martin Evans and Matthew Kaufman, as well as Gail Martin, independently established «true» pluripotent ESCs by directly cultivating mouse embryos that circumvented the tumourigenic stage of teratocarcinomas⁴. In the following years, various groups, mainly in the UK and the United States, worked with murine ESCs. In Germany, two groups established mouse ESC lines and characterized the cells by *in vitro* differentiation, teratocarcinoma formation and karyotype analyses⁵.

Research on mouse ESCs during the 1980s and early 1990s concentrated on characterization of their properties, the optimization of *in vitro* propagation, differentiation into derivatives of all three germ layers, and development of strategies for genetic modification by gain-of-function and loss-of-function (gene targeting)⁶. But only the derivation of the first human embryonic stem cell (hESC) lines by James Thomson and co-workers at the end of 1998⁷ attracted significant public attention. The prospect of generating specialized human cells from an unlimited cell source raised hopes of therapies for hitherto incurable human diseases. However, a fundamental drawback of hESC derivation, the necessity to destroy early human embryos at the blastocyst stage, raised ethical concerns and heavily affected the public debate on the use of hESCs.

To be pointed out is that research on embryonic stem cells developed independently of the discovery of adult hematopoietic stem cells by James Till and Ernest McCulloch in the early 1960s, when hematopoi-

⁴ M.J. EVANS - M.H. KAUFMAN, *Establishment in Culture of Pluripotential Cells from Mouse Embryos*, in «Nature» 291, 1981, pp. 154-156.; G. MARTIN, *Isolation of a Pluripotent Cell Line from Early Mouse Embryos Cultured in Medium Conditioned by Teratocarcinoma Stem Cells*, in «Proceedings of the National Academy of Sciences», 78, 1981, pp. 7634-7638.

⁵ A.M. WOBUS - H. HOLZHAUSEN - P. JÄKEL - J. SCHÖNEICH, *Characterization of a Pluripotent Stem Cell Line Derived from a Mouse Embryo*, in «Experimental Cell Research», 152, 1984, pp. 212-219.; T.C. DOETSCHMAN et al., *The in vitro Development of Blastocyst-derived Embryonic Stem Cell Lines: Formation of Visceral Yolk Sac, Blood Islands and Myocardium*, in «Journal of Embryology & Experimental Morphology», 87, 1985, pp. 27-45.

⁶ A.M. WOBUS - K.R. BOHELER, *Embryonic Stem Cells*.

⁷ J.A. THOMSON et al., *Embryonic Stem Cell Lines Derived from Human Blastocysts*, in «Science», 282, 1998, pp. 1145-1147.

etic stem cells were characterized as multipotent stem cells capable of self-renewal and differentiation⁸. Thus, contrary to claims by opponents of hESC research, embryonic stem cell research is not a mere product of progress in adult stem cell research; rather, it has different roots.

II. STEM CELL RESEARCH IN GERMANY WITHIN THE PRIORITY PROGRAM 1109 OF THE GERMAN RESEARCH FOUNDATION 2001-2007

Germany was among those countries in which the derivation of the first hESC lines in 1998 rapidly caused heated debate on stem cell research. Scientists, scientific organisations, and the public discussed the potential and challenges, but also the ethical and possible legal implications, of hESC research. In spring 1999, the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG) began its first consultations on stem cell research with cell biologists, stem cell researchers and physicians. The aim of this initiative was to induce synergistic effects by concentrating different stem cell research activities into a specific research program. A series of meetings followed with the purpose of establishing a Priority Program (*Schwerpunktprogramm*) for stem cell research. In March 2000, a first project proposal *Embryonic and tissue-specific stem cells – Regenerative systems for cell and tissue repair* was submitted, and in June 2000 the Senate of the DFG recommended the funding of a Priority Program with the following areas prioritized for project applications: (1) genetics and cell biology of the asymmetric division of somatic stem cells including model organisms; (2) analysis of the developmental capacity of (mouse) ESCs and integration of differentiated cell types into animal models; (3) analysis of somatic stem cells with emphasis on stem cell plasticity; and (4) germ cells and mechanisms of reprogramming. To be noted is that the use of hESCs and the legal situation of working with hESCs were at that time still under discussion in Germany. In January 2001, the funding of the first 16 projects of the DFG Priority Program 1109 started, and in the following years a total of 26 projects were supported in 2- or 3-year funding periods between 2001 and 2007⁹.

⁸ J.E. TILL - E.A. McCULLOCH, *A Direct Measurement of the Radiation Sensitivity of Normal Mouse Bone Marrow Cells*, in «Radiation Research», 14, 1961, pp. 213-222.

⁹ A.M. WOBUS, *Stem Cell Research within the Priority Program 1109 of the German Research Foundation, 2001-2007*, in «Cells Tissues Organs», 188, 2008, pp. 6-8.

III. HUMAN ESC RESEARCH IN GERMANY ACCORDING TO THE STEM CELL ACT

In 1999, the DFG issued a first statement on research involving hESCs, followed by a second statement on *Research with Human Embryonic Stem Cells* presented to the public in May 2001. In parallel, this topic was discussed by the German Federal Parliament's Commission of Inquiry into *Prospects and Risks of Modern Medicine* and by a newly established National Ethics Council, which submitted a joint report at the end of 2001. After a first parliamentary debate in December 2001, the Federal Government implemented the parliamentary resolution in January 2002. The legislative process resulted in the Stem Cell Act (Stammzellgesetz, StZG), which became effective on 1 July 2002. The Embryo Protection Act, which prohibits the use of human embryos for research purposes, remained in force, with the consequence that the establishment of hESCs is prohibited in Germany.

The Stem Cell Act prohibits the import and use of hESCs as a basic principle, but at the same time defines circumstances under which hESCs may be imported into Germany and used for research purposes. The following requirements must be fulfilled with respect to the hESC lines in order to obtain a license for the import and use of hESCs: (1) the embryonic stem cells must have been derived before 1 January 2002 in the country of origin in accordance with its relevant national legislation there; (2) the embryos from which they were derived have been produced by medically assisted *in vitro* fertilisation to induce pregnancy and were definitely no longer used for this purpose, and that there is no evidence that this was due to reasons inherent to the embryos themselves; (3) no compensation or other payment in kind has been granted or promised for the donation of embryos for stem cell derivation.

Furthermore, the Stem Cell Act also defines the prerequisites that must be met by the research project in which the use of hESC lines is envisaged: (1) the research project must serve goals of a premium value for the acquisition of scientific knowledge; (2) results from appropriate preliminary experiments involving animal or non-ES human cells must exist to provide pre-clarification of the research project; and (3) the intended advance in scientific knowledge must require the use of hESCs and cannot be obtained by using a different cell type.

One rationale for these restrictions was to prevent research projects undertaken in Germany from causing further embryo destruction outside Germany.

The Central Ethics Commission (Zentrale Ethik-Kommission für Stammzellenforschung, ZES) is charged with the task of reviewing and evaluating research projects involving hESCs submitted by German scientists to the licensing authority, the Robert Koch Institute (RKI). The ZES must determine whether the research proposals comply with the regulations set forth in the Stem Cell Act, and it must submit a written opinion to the RKI. The first license for the import and use of hESCs was granted to Oliver Brüstle, of the University of Bonn, in December 2002. The project immediately became part of the DFG-funded Stem Cell Priority Program.

However, the Stem Cell Act had several shortcomings, two of which became apparent soon after its enforcement. First, from 1998 onwards, important advances were achieved in the hESC field, for example with respect to the derivation and cultivation of hESCs. The number of newly established hESCs continually increased, and derivation techniques steadily improved. Because of the cut-off date originally defined in the Stem Cell Act (1 January 2002), German researchers were initially allowed to work with only 21 hESC lines listed in the registry of the National Institutes of Health (NIH) of the United States. These hESC lines were not standardized and, in addition, they were potentially contaminated by animal cells and proteins. In fact, the so-called NIH hESC lines were established under non-standardized conditions in the presence of animal sera and supplements and cultured on animal feeder cells.

Consequently, German hESC research became increasingly restricted, and international cooperations proved difficult owing to the availability of only a few hESC lines in Germany. Second, it was not entirely clear from the wording of the Stem Cell Act whether a German researcher working abroad with hESCs or participating in international projects would be in breach of the Stem Cell Act. Consequently, it could not be excluded that German researchers would be confronted with legal problems in Germany as a consequence of international collaborations. Therefore, the German Research Foundation and the German academies (Berlin-Brandenburg Academy of Sciences; German Academy of Natural Sciences Leopoldina) independently published statements

on human ESC research and proposed an amendment to the Stem Cell Act. Specifically, a more recent cut-off date for the import and use of newly established hESC lines and the abandonment of penalties for violation of the Stem Cell Act have been recommended. After hearings and a parliamentary discussion, the amendments to the Stem Cell Act were adopted by the parliament and enacted in August 2008. According to the amended Stem Cell Act, a new cut-off date (1 May 2007) was set for the import of hESC lines. Moreover, the regulations concerning the area of application of the Stem Cell Act were modified so that it became unambiguously clear that only hESC research performed within Germany was subject to the regulations. However, the request of the DFG and the Academy of Leopoldina to allow the use of hESCs not only for research purposes but also for commercial and routine applications was not granted.

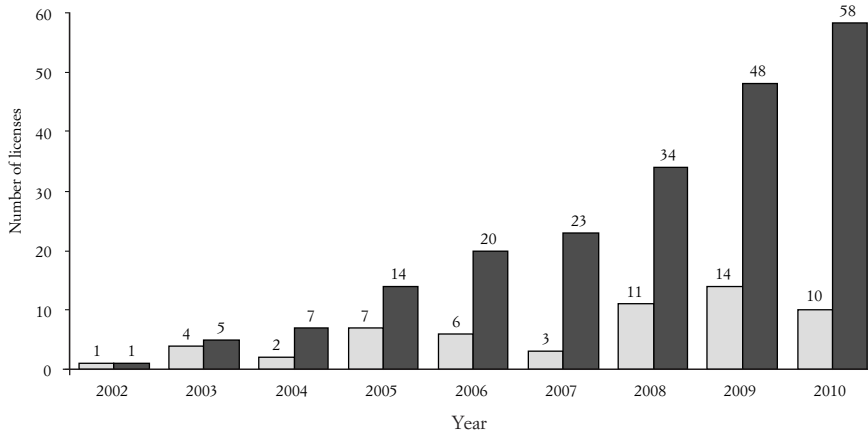
IV. THE PRESENT STATE OF hESC RESEARCH IN GERMANY

According to the Stem Cell Act, scientists who intend to work with hESCs in Germany must apply for a license. For each application, the Central Ethics Committee (ZES) examines whether the research proposal is in accordance with the regulations set forth by the Stem Cell Act. After review by the ZES, an opinion is submitted to the licensing authority (RKI), which must make the final decision on the application. Although the RKI is not constrained by the ZES's opinion, in the past there has been agreement between RKI and ZES on all applications. To date (December 2010), a total of 58 licenses for import and/or use of hESCs have been granted in Germany (see figure 1), while two proposals have been rejected and two projects have been discontinued.

At present, hESC research is being performed in 32 German institutions by 44 research groups. While many hESC projects are undertaken at universities and in the research groups of scientific organizations such as the Max-Planck-, Fraunhofer- and Helmholtz-Societies, some hESC research is also conducted by companies and scientific agencies (see table 1).

The geographical distribution of the institutions performing hESC research licensed by the RKI demonstrates that North-Rhine Westphalia with 20 projects occupies the leading position in German hESC research.

Figure 1. Licences for import and/or use of hESCs



Number of Licences for import and/or use of hESCs granted by the Robert Koch Institute (RKI) after approval by the Central Ethics Committee for Stem Cell Research (ZES) in the given years (light grey bars) and in total by the end of each year (dark grey bars). Two applications have been rejected both by ZES and the RKI to date. Four applications were pending at the end of 2010.

Source: Robert Koch Institute, www.rki.de

This may be due to the constant support given to stem cell research by the North-Rhine Westphalian government. Moreover, Berlin and Lower Saxony are regions in which several groups and institutions are engaged in research with hESCs, while other federal states play only a minor role (see fig. 2).

After the successful reprogramming of somatic cells to human-induced pluripotent stem cells (hiPSCs) by Shinya Yamanaka¹⁰ and others¹¹ and after the amendments to the Stem Cell Act (see above), researchers in Germany broadened their research activities. Projects in which hESCs and hiPSCs were compared in regard to their characteristics and dif-

¹⁰ K. TAKAHASHI - S. YAMANAKA, *Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors*, in «Cell», 126, 2006, pp. 663-676; K. TAKAHASHI et al., *Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors*, in «Cell», 131, 2007, pp. 861-872.

¹¹ J. YU et al., *Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells*, in «Science», 318, 2007, pp. 1917-1920.

Table 1. *German institutions using hESCs for research*

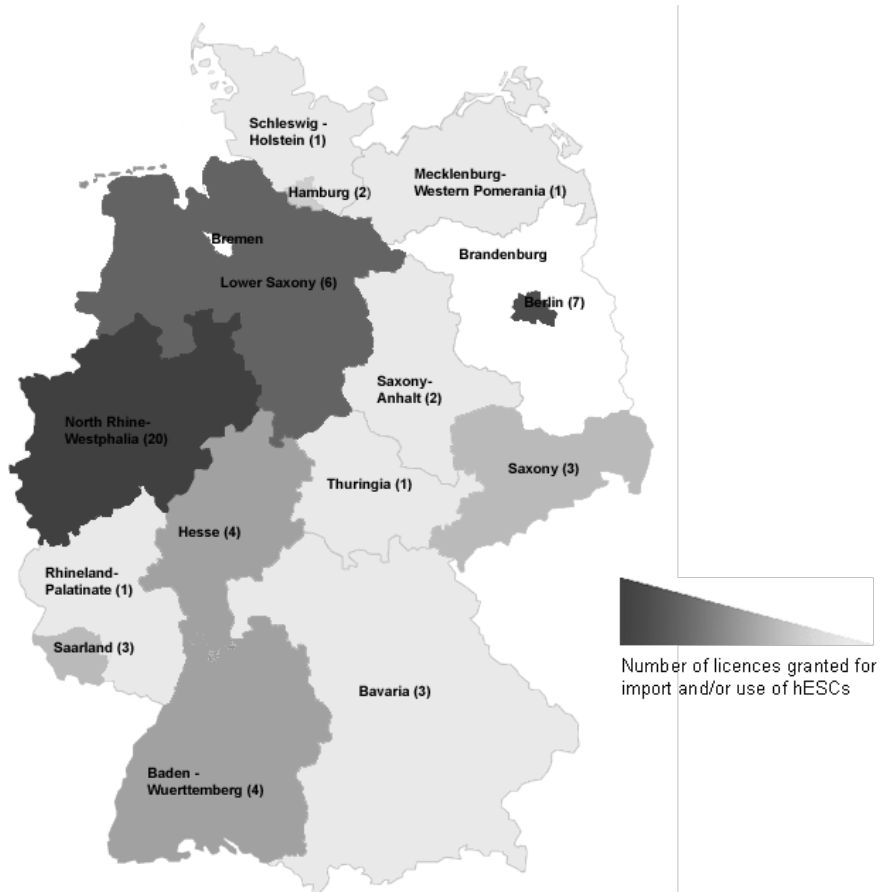
Institution	Number of Research Groups	Number of Licences
<i>Research Organizations</i>		
Max Planck Society	3	7
Fraunhofer Society	2	3*
Helmholtz Society (MDC Berlin)	3	3
<i>Universities</i>		
22 Universities and Medical Schools (Berlin, Bonn, Köln, Dortmund, Dresden, Düsseldorf, Essen, Frankfurt, Freiburg, Giessen, Göttingen, Halle, Hamburg, Hannover, Heidelberg, Jena, Konstanz, München, Münster, Rostock, Tübingen, Würzburg)	31	40
<i>Companies</i>		
ProteoSys AG (Mainz)	1	1
Miltenyi Biotec GmbH (Bergisch- Gladbach)	1	1
Life & Brain GmbH (Bonn)	1	1
<i>(Private) Non-Profit Organizations</i>		
ZIP gGmbH (Kiel)	1	1
<i>Governmental Agencies</i>		
Paul Ehrlich Institute (Langen)	1	1
Total: 32 Institutions	44	58

The number of groups working with hESCs at these institutions and the number of licenses granted by the Robert Koch Institute until the end of 2010 are shown.

* One research project was completed in December 2010.

Source: Robert Koch Institute, www.rki.de

Figure 2. *Regional distribution of projects involving hESCs among the federal states of Germany*



Shown is the number of projects approved by the Robert Koch Institute (RKI) by the end of 2010. All projects also received approval from the Central Ethics Committee for Stem Cell Research (ZES).

Source: Robert Koch Institute, www.rki.de

differentiation properties were initiated, and newly established cell lines were imported into Germany for use in research projects. As shown in Figure 3, from 2007 onwards, research projects in which only hESCs were used and projects involving both hESCs and hiPSCs were initiated. Moreover, hESC lines derived between the old and new cut-off dates (between 1 January 2002 and 1 May 2007) were approved for import to Germany and for use in research projects (see table 2).

Before the amendment of the Stem Cell Act, only 20 hESC lines derived before the original cut-off date (1 January 2002) were approved by the licensing authority for import and use. Strikingly, in only two years after the amendment of the Stem Cell Act, licenses were granted for the import and use of an additional 23 hESC lines derived between 1 January 2002 and 1 May 2007. This reflects the strong interest of researchers in using these «new» hESC lines, and confirms the necessity to implement the much-discussed modifications to the Stem Cell Act.

Research projects involving hESCs in Germany were (and still are) financially supported by the German Research Foundation (DFG, table 3), the Federal Ministry of Education and Research (BMBF, table 4), the European Union in framework 6 and 7 programs, and private donors.

When considering the funding practice of the DFG and the BMBF, to be noted is that the funding of hESC research accounts for only a low percentage of the total funding of all stem cell research projects. The DFG supported all stem cell projects (including embryonic and adult human and animal stem cells) between 2000 and 2009, with a total amount of at least 93,89 million EUR, while projects involving hESCs received funding of only 5,11 million EUR, which is only 5.4% of the total amount spent for research on all stem cell types (see table 3).

At present, the DFG is supporting a number of ambitious stem cell programs. After the Priority Program 1109 was completed in 2007, the new stem cell Priority Program 1356 on *Pluripotency and Reprogramming* was begun. This program was launched in response to the breakthrough reprogramming studies by Shinya Yamanaka¹². It funds

¹² K. TAKAHASHI - S. YAMANAKA, *Induction of Pluripotent Stem Cells*; K. TAKAHASHI et al., *Induction of Pluripotent Stem Cells*.

Table 2. *hESC lines approved for import to Germany and for use in research projects by the end of 2010*

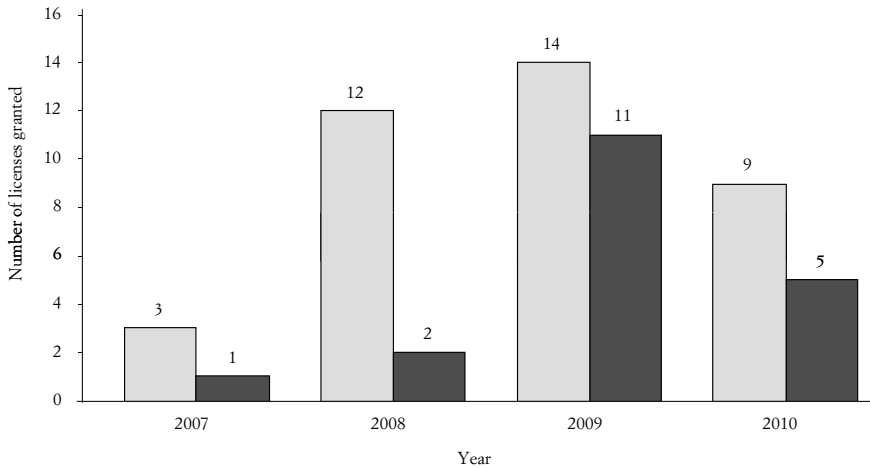
Provider	Number of hESC lines	Name of hESC lines
BresaGen, Inc. (Division of Novocell), Athens, GA, USA)	3	BG01, BG02, BG03
Cellartis AB, Göteborg, Sweden	7	SA01, SA02, SA121 , SA167 , SA181 , SA348 , SA461
ES Cell International Ltd, Singapore	6	ES01, ES02, ES03, ES04, ES05, ES06
Harvard University, Cambridge, MA, USA	7	HUES1, HUES2, HUES4, HUES6, HUES7, HUES8, HUES10
Karolinska Institute, Stockholm, Sweden	3	HS181 , HS401 , HS415
Kyoto University, Kyoto, Japan		KhES-1, KhES-2, KhES-3
Centre for Life, Newcastle Fertility Centre, Newcastle, UK	2	NCL-3 , NCL-4
University of Sheffield, Sheffield, UK	3	Shef-1 , Shef-2 , Shef-3
Technion, Israeli Institute of Technology, Haifa, Israel	4	TE03, TE04, TE06, TE07
Wisconsin Alumni Research Foundation (WARF), WiCell, Madison, WI, USA	5	WA01, WA07, WA09, WA13, WA14

Twenty hESC lines were derived before the original cut-off date stipulated in the Stem Cell Act (1 January 2002), while 23 cell lines (in bold) were derived between 1 January 2002 and 1 May 2007 (the cut-off date as determined by the amended Stem Cell Act of August 2008). Note that the validation whether a specific hESC line meets the criteria of article 4 of the German Stem Cell Act is only performed after an application for import and use of this hESC line has been filed. It is estimated that a large number of additional hESC lines might also meet the criteria of the Stem Cell Act.

Source: Robert Koch Institute, www.rki.de

research on genetic and epigenetic networks that control pluripotency, induction of pluripotency by nuclear reprogramming, and mathematical modelling of pluripotency. Besides this program, the DFG is supporting further stem cell research projects submitted as single project proposals and undertaken within other programs.

Figure 3. Use of hESCs and hiPSCs in research approved by the Robert Koch Institute (RKI) and the Central Ethics Committee for Stem Cell Research (ZES) from 2007 to 2010



Shown are the numbers of hESC research licenses granted in recent years (light grey bars) and the numbers of approved hESC projects which also involve the use of hiPSCs (dark grey bars).

Source: Robert Koch Institute, www.rki.de

One of these programs is called *Regenerative Therapies: From Cells to Tissues to Therapies* pursued at the Center for Regenerative Therapies in Dresden (CRTD). The program started in 2006 and comprises working groups at the University of Technology (TU) Dresden, the Biotechnology Center, the Max-Planck-Institute on Cell Biology and Genetics (MPI-CBG), the Max-Bergman Center for Biomaterials, and the Medical Theoretical Center. The main concerns of the research projects are haematology, oncology, immunology of stem cells, diabetes, neurodegeneration, hard tissue replacement and cardiovascular diseases, while only a few projects are working with human ESCs.

In 2006, the CRTD was nominated a «Cluster of Excellence» and received additional support from the DFG within the DFG Excellence Center Engineering the Cellular Basis of Regeneration. Moreover, stem cell groups at the Hanover Medical School obtained funding within the Excellence Cluster from Regenerative Biology to Reconstruction (REBIRTH).

Table 3a. *Number of applications and approvals for the funding of projects in the field of stem cell research in Germany by the German Research Foundation (2000-2009)*

DFG-funded stem cell projects including research on hES cells					
Year	Applications		Approvals		Funding (total)
	Single Applications	Projects within framework programs*	Single Applications	Projects within framework programs*	Approved (Million €)
2000	46	12	22	6	3,11
2001	79	9	36	8	4,89
2002	93	10	37	7	3,94
2003	99	13	42	9	5,96
2004	91	14	35	12	4,25
2005	115	13	60	9	11,75
2006	112	26	41	15	9,40
2007	107	15	47	11	9,94
2008	152	19	73	14	20,05
2009	132	20	69	16	20,60

* The number correlates with the number of different framework programs.

Source: DFG

The Federal Ministry of Education and Research (BMBF) has supported a number of initiatives in regenerative medicine, such as the Organ Substitution and Cell-Based Regenerative Medicine programs, and research on Pluri- and Multipotent Stem Cells (see table 4). In addition, two Translational Centers for Regenerative Medicine (TRM) at Berlin and Leipzig are supported with 7.5 million EUR each. A total amount of at least 270 million EUR has been spent by the BMBF for basic and applied research in the field of regenerative medicine, including stem cell research. While most projects deal with human or animal somatic stem or progenitor cells, animal ES cells and induced pluripotent stem (iPS) cells, only a small proportion (7.8%) of projects involve hESCs.

A new reference centre for stem cell research, the Center for Applied Regenerative Developmental Technologies (CARE), has been recently founded in Münster. Over the next decade, this centre will be funded with a total of 80 million EUR by the *Land* North Rhine-Westphalia (60 million) and the BMBF (20 million). The Max-Planck Society will also contribute funding.

Table 3b. *Number of applications and approvals for the funding of projects in the field of hESC research in Germany by the DFG (2000-2009)*

Year	DFG-funded projects on hESCs on				
	Applications		Approvals		Funding (total)
	Single Applications	Projects within framework programs*	Single Applications	Projects within framework programs*	Approved (Million €)
2000					
2001					
2002	1	1	1	1	0,22
2003	1	0	1	0	0,23
2004	4	2	3	2	0,51
2005	4	1	3	1	0,68
2006	6	1	2	1	0,20
2007	4	0	2	0	0,46
2008	7	3	3	2	1,06
2009	14	0	4	0	1,75

* The number correlates with the number of different framework programs.

Source: DFG

Table 4a. *Project applications submitted to the Federal Ministry of Education and Research (BMBF) within specific calls of unit 615 (Health Research)**

Announcement (Year)	1999	2004	2007	2008	2009
Topic of research (main focus)	Organ substitution	CRM1	PMSC1	CRM2	PMSC2
Number of projects	182	332	123	251	104
Number of grants	32	47	29	57	22
Funding applied for (Million €)	79	96	48	82	41
Funding granted (Million €)	10	12	9	19	6
Funding period	2001-06	2005-09	2008-12	2009-12	2010-13

* Research programs: Organ substitution = Biological substitution of organ functions. Given are the number of project applications, grants and the amount of funding in Millions of EUR.

Source: BMBF

Table 4b. *Funding of stem cell research within specific calls of the BMBF in Millions of EUR**

Year	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Organ substitution	1	2,6	3,4	2,2	0,6	0,1				
CRM1					0,8	3,9	3,9	3	0,4	
CRM2									1,9	5,8
PMSC1								0,4	2,5	2,6
PMSC2										0,7
Sum	1	2,6	3,4	2,2	1,4	4	3,9	3,4	4,8	9,1

Source: BMBF

Table 4c. *Cell types used in research programs funded by the BMBF. Shown is the funding in Million EUR**

	Organ substitution (1999)	CRM1 (2004)	PMSC1 (2007)	CRM2 (2008)	PMSC2 (2009)	Sum
Human ES cells	3	15	0	3	0	21
Animal ES cells	8	16	0	4	0	28
Human somatic stem and progenitor cells	16	28	3	32	15	94
Animal somatic stem and progenitor cells	14	15	6	39	2	76
Germ-line derived cells	0	0	6	11	0	17
iPS cells	0	0	14	11	9	34

Total: ~ 270 Million EUR

* Abbreviations: CRM1 = Cell-based regenerative medicine, Phase 1; CRM2 = Cell-based regenerative medicine, Phase 2; PMSC1 = Pluri- and multipotent stem cells, Phase 1; PMSC2 = Pluri- and multipotent stem cells, Phase 2; iPS cells = induced pluripotent stem cells.

Source: BMBF

V. PLURIPOTENT STEM CELL RESEARCH IN GERMANY IN COMPARISON TO INTERNATIONAL RESEARCH

According to the Stem Cell Act, all projects that involve hESCs must be listed in a public registry located on the web pages of the Robert Koch Institute¹³. This register allows the identification of research areas and topics of hESC research in Germany. Most of the projects deal with basic research and pursue the following aims: 1. Establishment and optimization of *in vitro* cultivation, purification and genetic modification of hESCs, 2. Analysis of molecular regulation of pluripotency and characterization of signalling networks controlling pluripotency, 3. Differentiation of hESCs towards differentiated derivatives, such as neural, cardiac, liver, blood, bone, lung or pancreatic cells, and 4. Studies using hESCs in comparison to other pluripotent stem cells, such as iPS cells or spermatogonial stem cells.

As mentioned above, out of the more than 1300 hESC lines established by the end of 2010 (see table 5), usable in Germany are only those cell lines that have been established before May 2007 and comply with the other stipulations of the Stem Cell Act (see table 2). However, the Stem Cell Act also precludes the import and use of cell lines derived from embryos no longer used for reproductive purposes for reasons inherent to the embryos themselves. Not allowed, therefore, is the import and use of human ESCs derived from IVF embryos analysed by preimplantation genetic diagnosis (PGD) for potential hereditary diseases. Consequently, the at least 166 hESC lines that carry mutations causative of 46 inheritable human diseases («disease-specific hESC lines», table 6) currently existing cannot be used for research in Germany. However, it is generally accepted that research using these hESC lines may be valuable to gain deeper understanding of pathogenesis mechanisms and to identify novel diagnostic and therapeutic strategies for the respective diseases.

Since 2004, scientists involved in hESC research in Germany have published a number of papers. However, by the end of 2009, only 23 original papers describing experimental work involving hESCs had been published by German researchers in English-language journals indexed in PubMed. Only four of these papers resulted from projects

¹³ http://www.rki.de/DE/Content/Gesund/Stammzellen/Register/register_node.html

Table 5. *Overview of hESC lines publicly reported by the end of 2010*

Country	Number of hESC lines	Number of institutions	Number of hESC lines published in the scientific literature
Australia	69	4	25
Belgium	47	3	29
Brazil	1	1	0
Canada	6	2	4
China (incl. Taiwan)	270	16	261
Czech Republic	7	1	4
Denmark	31	4	14
Finland	14	2	12
France	24	5	14
India	17	4	7
Iran	10	1	10
Israel	30	3	28
Japan	3	1	3
Korea	37	5	35
Netherlands	4	1	4
Russia	16	2	5
Singapore	15	2	15
Spain	26	4	22
Sweden	90	3	45
Switzerland	4	2	1
Thailand	1	1	1
Turkey	18	1	16
United Kingdom	50	9	35
United States	516	22	225
Total (24 Countries)	1306	99	815

The numbers of institutions at which hESC lines were derived and of hESC lines reported in peer-reviewed publications are also given. Cell lines derived by the Reproductive Genetics Institute (Chicago IL) and partially distributed by Stemride International Limited (London, UK) have been assigned to the United States. hESC lines from ES Cell International Ltd. have been assigned to Singapore.

Source: Ref. 14 and unpublished data

funded by the DFG. When considered in the international context and compared to other countries, primarily the United States and the United Kingdom, the 23 original publications from hESC studies performed in Germany is only a small number. To date, German hESC research has accounted for only 1.7% of total publications on hESCs worldwide. Other countries, such as Israel, South Korea, Singapore, China, Australia, Japan, Canada and Sweden, are much more produc-

Table 6. *hESC lines with genetic disorders causative of inheritable human diseases («disease-specific» hES cell lines) as reported in the scientific literature by the end of 2010*

Genetic Disorder	Number of hESC lines
Adrenoleukodystrophy	1
alpha-Thalassaemia	2
Alport Syndrome	2
Androgen Insensitivity Syndrome (AIS)	2
beta-Thalassaemia	8
Charcot-Marie-Tooth syndrome	5
Cystic fibrosis (CF)	21
Dystrophia myotonica type 1 (DM1)	8
Fabry Syndrome	1
Facio Scapulo Humeral (FSH) muscular dystrophy	10
Familial adenomatous polyposis (FAP)	3
Familial breast cancer (BRCA1)	1
Familial breast cancer (BRCA2)	1
Fanconi anaemia	1
Fragile X syndrome (FX)	15
Gaucher's disease	1
Hemophilia A	1
Huntington's disease (HD)	15
Infantile Neuroaxonal Dystrophy	1
Marfan syndrome (MFS)	4
Multiple endocrine neoplasia type 1	2
Multiple endocrine neoplasia type 2	4
Multiple exostoses type 2	1
Muscular dystrophy, type Becker	2
Muscular dystrophy, type Duchenne	6
Muscular dystrophy, type Emery Dreifuss	4
Myotonic dystrophy (unspecified)	2
Myotubular myopathy (MTM)	2
Neurofibromatosis	2
Neurofibromatosis type 1	7
Ocular albinism	2
Osteogenesis imperfecta type 1	1
Pelizaeus-Merzbacher Disease (PMLD)	1
Polycystic Kidney Syndrome	1
Popliteal Pterygium Syndrome (PPS)	1
Saethre-Chotzen Syndrome (Acrocephalosyndactyly Type 3, ACS III)	1
Sandhoff disease	3
Sickle cell anaemia	3
Spastic paraplegia type 4	1
Spinal muscular atrophy type 1 (SMA1)	2

Spinocerebellar Ataxia Type 2 (SCA2)	1
Spinocerebellar Ataxia Type 7 (SCA7)	1
Torsion dystonia (DYT1)	4
Treacher Collins-Franceschetti Syndrome (TCOF), affected	2
Tuberous sclerosis	4
Von Hippel-Lindau (VHL) Syndrome	3
Total: 46 genetic disorders	166

Source: Ref. 14 and unpublished data

tive in this research field (see table 7)¹⁴. Moreover, the impact of hESC research papers from Germany is lower than that of papers produced in other countries. For example, research papers from the United States reporting experimental work involving hESCs and published between 2004 and 2008 had an average citation frequency of 48.0 citations per paper in 2009, and papers from Israel, Canada and the UK published in the same period were cited an average of 42.8, 35.9 and 36.7 times in 2009, respectively. By contrast, hESC research papers from Germany were cited an average of 21.6 times in 2009. The reasons for the lag of German hESC research have not yet been investigated in detail. Reservations in regard to hESC research by a part of the policy community and the general public, the relatively low funding, and the restrictions in view of the commercial use of hESCs may have contributed to the relatively weak international position of German hESC research.

By contrast, research involving hiPSCs was warmly welcomed even by those parts of the public and the policy community that regarded hESC research critically for ethical, religious or ideological reasons. Human iPSC research is considered to be «free» of ethical problems because no embryo destruction is necessary to provide human pluripotent stem cells. However, Germany's contributions in this scientific field were still limited by the end of 2009. Whereas research groups in the United States and Japan had published 60 and 16 original research papers on hiPSCs, respectively, only 3 publications came from German research groups (see table 8). It will be interesting to see whether Germany will strengthen its position in this novel research field within the next years, or whether the scientific backlog in ESC research will have a negative impact on the development of hiPSC research in Germany.

¹⁴ P. LÖSER et al., *Human Embryonic Stem Cell Lines and Their Use in International Research*, in «Stem Cells», 28, 2010, pp. 240-246.

Tab. 7. *Numbers of hESC research papers published between 1998 and 2009*

Country	Number of hESC papers	% of total hESC papers
Australia	52	3,8
Austria	1	0,1
Belgium	17	1,2
Brazil	1	0,1
Canada	40	2,9
China	69	5,0
Czech Republic	7	0,5
Denmark	10	0,7
Estonia	1	0,1
Finland	15	1,1
France	18	1,3
Germany	23	1,7
Hungary	2	0,1
India	17	1,2
Iran	10	0,7
Israel	81	5,9
Italy	12	0,9
Japan	50	3,6
Korea	70	5,1
Netherlands	16	1,2
Portugal	1	0,1
Romania	1	0,1
Russia	6	0,4
Singapore	71	5,2
Spain	18	1,3
Sweden	54	3,9
Switzerland	8	0,6
Thailand	1	0,1
Turkey	5	0,4
United Kingdom	112	8,1
United States	588	42,7
Total	1377	100

Given are the number of original research papers published in peer-reviewed English language journals and the share of work from a country in the total number of hESC papers. According to published work, hESC research is being carried out in at least 31 countries. Assignment of a paper to a country has been according to the corresponding author's address. Only work involving experimental use of hESCs has been included, whereas reviews, comments and papers reporting previously published methods or legal and ethical aspects of hESCs have been omitted.

Source: Ref. 14

Table 8. *Number of papers on derivation and/or experimental use of hiPSCs published from 2007 to 2009 in peer-reviewed English language journals*

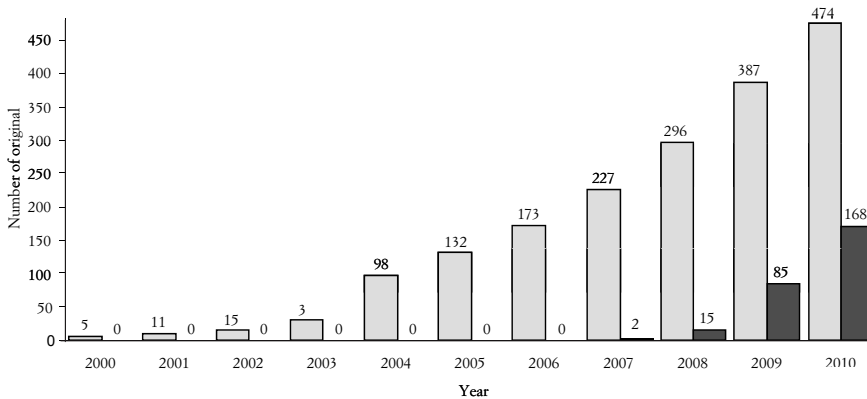
Country	Number of hiPSC papers	Share in total hiPSC papers
Canada	3	2,9
China	7	6,9
France	1	1,0
Germany	3	2,9
Iran	1	1,0
Israel	1	1,0
Japan	16	15,7
Spain	5	4,9
Switzerland	1	1,0
United Kingdom	4	3,9
United States	60	58,8
Total	102	100

Given are the number of original research papers and the share of work from a country in the overall number of hESCs papers. According to published work, hiPSC research was carried out in at least 11 countries by the end of 2009. Note that papers that were published online 2009, but appeared in print in 2010 are not included.

Source: Ref. 14 and unpublished data

One reason advanced by the proponents of hiPSC research who object to hESC research is that hiPSCs may very soon replace hESCs in research. However, a comparison of international activities in both research fields does not support this assumption (see figure 4). In 2010, the number of both peer-reviewed hESC and hiPSC research papers published in English-language journals increased further. Notably, a large proportion of papers on hiPSCs also involved the parallel use of hESCs either for comparative purposes or to investigate a scientific question in this cell type before the methods are applied to hiPSCs. Therefore, by the end of 2010 both research fields existed independently, had been further extended and partially overlapped.

Figure 4. *Numbers of papers reporting derivation and/or experimental use of hESCs (light grey bars) and hiPSCs (dark grey bars) as published by 10 December 2010 in peer-reviewed English language journals*



Note that the numbers for 2010 are preliminary and also include papers that were published online in 2010 but will appear as printed papers only in 2011.

Source: Ref. 14 and unpublished data

VI. CONCLUSION

Research on hESCs is an internationally well-accepted and rapidly expanding research field. Since the first publications on hESCs in 1998, research on hESCs has become internationally established as one of the new scientific disciplines now developing exponentially. Worldwide, a constant increase is apparent both in the number of newly established hESC lines and in the number of publications on those cells.

In Germany, hESC research is regulated by the Stem Cell Act of 2002 and its amendment of 2008. On the basis of the Stem Cell Act, research with hESCs is possible in Germany if the cells have been established before the cut-off date of May 2007, if the projects serve research goals of premium value, and if there is no alternative to the use of hESCs. When comparisons are made with other countries, specific conditions regulating hESC research in Germany should be considered: (1) human ESC research projects must be licensed in a two-step procedure that involves a governmental authority and an independent ethics committee;

(2) only research projects involving hESCs, but not the commercial use of these cells, are allowed by the Stem Cell Act; (3) although hESC projects are funded by German agencies, the level of funding is relatively low in comparison with other countries, specifically the United States or countries in the Asian-Pacific area. Consequently, the number of publications published on hESCs by German scientists is relatively small, and at present, the same applies to publications on hiPSCs.

On the other hand, certain advances have been achieved in Germany on the basis of legally regulated hESC research. Specifically, progress in human ESC and iPSC research in Germany has been driven by the continuous initiatives and activities of German researchers and the support of scientific bodies. With respect to international developments, research on hESCs has further expanded, and research on both hESCs and hiPSCs has been performed in parallel. At present, it is an open question whether hiPSC research will replace studies with hESCs in the future.