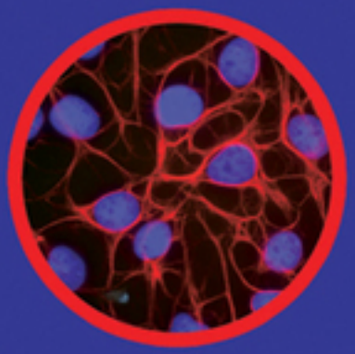
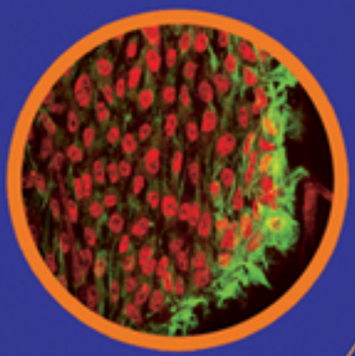


# Human STEM CELL Technology and Biology



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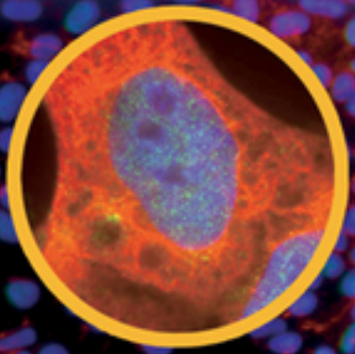

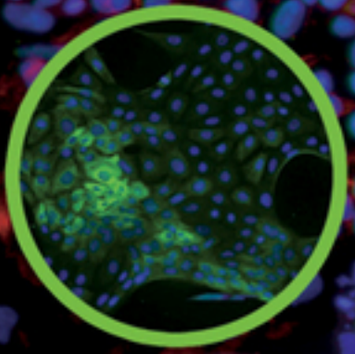


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# Human Stem Cell Technology and Biology

## A RESEARCH GUIDE AND LABORATORY MANUAL

*Edited by*

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# Foreword

In recent years interest in stem cells has become intense, not only within the scientific and medical communities but also amongst politicians, religious groups and ethicists. Stem cells offer tremendous potential to alleviate human disease, yet opinions diverge about how that potential can best be realized. In *Human Stem Cell Technology and Biology: A Research Guide and Laboratory Manual*, Gary Stein and his colleagues provide a timely introduction to stem cells and step-by-step protocols for working with them in the laboratory. The book is based on the highly successful practical courses provided at the University of Massachusetts Center for Stem Cell Biology & Regenerative Medicine.

This book achieves two important goals: to provide reliable laboratory protocols for culturing pluripotent stem cells and to present current perspectives and applications of stem cells. It begins with a historical account of stem cell research, explaining the different types of stem cell that have been characterized. The second section describes techniques for culturing, maintaining and characterizing pluripotent stem cells, including the pros and cons of different methods. The final section provides an in-depth perspective on the current status of the stem cell research field. Special features include downloadable protocols and narrated videos that present the principle techniques for human pluripotent cell culture.

*Human Stem Cell Technology and Biology: A Research Guide and Laboratory Manual* will greatly benefit both established investigators and newcomers in the stem cell field. It helps to demystify and explain the different types of pluripotent cell and will be an important tool for stem cell research for years to come.

Fiona M. Watt

*Cambridge, Massachusetts*
*January 2010*

# Preface

*Human Stem Cell Technology and Biology: A Research Guide and Laboratory Manual* was developed to serve two distinct but important functions: to provide laboratory techniques and protocols that have been tested and proved to be effective for culture of pluripotent stem cells, and to present current perspectives and applications of stem cells by the leaders in the field. The first section of the text begins with an overview of the research contributions that established the foundation for current initiatives in stem cell research and goes on to describe the best methods for researching and obtaining pluripotent cell lines: both blastocyst derived human embryonic stem cells and reprogrammed pluripotent stem cells.

The second and third sections provide skills and techniques necessary for the culture, maintenance and characterization of pluripotent stem cells. These sections have been developed as a multimedia stem cell course in an effort to present the most comprehensive instruction possible, including the following features:

- **Videos demonstrating step-by-step laboratory protocols.** While reading the correct procedure is important to understanding technique, there is no substitute for watching the procedure done by an expert. To ensure this objective is met, laboratory procedures for the culture of human embryonic and induced pluripotent stem cells are presented in narrated video format via an accompanying CD/DVD and on the web at www.wiley.com/go/stein/human Look for the video camera icon to locate text associated with video format.

- **Online updates.** Electronic updates will be made available on a scheduled basis. Availability of the rapidly developing strategies and research protocols that support stem cell research will extend the "life" of the book.
- **Printable laboratory protocols.** The CD/DVD also contains PDF files of lab protocols to be printed and taken into the lab. In this way, the files can become the basis for a lab notebook to provide a seamless transition from reading the text to performing the experiments.

We recognize and appreciate that the stem cell field is rapidly evolving. Concepts and strategies are emerging that will advance understanding of genetic and epigenetic control for development, tissue renewal, and regenerative medicine. The challenges and opportunities are formidable and we are committed to providing online updates on a regular basis to maximize the effectiveness of this book.

Gary S. Stein
Maria Borowski
Mai X. Luong
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# Introduction

SECTION

# 1 INTRODUCTION TO PLURIPOTENT STEM CELLS: BIOLOGY AND APPLICATIONS

*Maria Borowski and Gary S. Stein*

Current advances in human stem cell research utilize the latest tools of cell biology, molecular biology, chemistry, biomedical imaging, genomics, proteomics, and bioinformatics. Pluripotent stem cells, such as human embryonic stem cells (hESCs) and reprogrammed cells (induced pluripotent stem (iPS) cells) are defined as cells with the capacity to proliferate indefinitely as well as differentiate into all specialized cells, tissues, and organs of the body. These cells have enormous potential to offer therapies for diseases that have not proven treatable by conventional strategies.

## HISTORICAL PERSPECTIVE

The exploration and utilization of cellular differentiation can be traced back more than half a century (Fig. 1.1). Visionary experiments from the 1950s provided a compelling foundation for two principal parameters of biological regulation. These studies established the concepts of pluripotency (as defined above) and epigenetic control, which is the transmission of regulatory information during cell division that is not encoded by DNA. At that time, developmental biologists were testing whether the process of cellular specialization or differentiation involved permanent changes in the DNA. Irreversible changes at the DNA level in a differentiated cell would prevent that cell's DNA from directing embryonic development. In a series of elegant experiments, Robert Briggs and Thomas King established a method for nuclear transfer, which John Gurdon subsequently used to transfer nuclei from frog intestinal cells to enucleated frog. These egg cells successfully divided, eventually developing into a tadpole.[1,2] These experiments demonstrated that changes to the DNA during differentiation were reversible and laid the groundwork for the discovery of pluripotent cells.

In mammals, work with embryonal carcinoma cells (malignant germ cell tumors) in the 1950s and 1960s established the presence of populations of mammalian cells that are capable of unlimited self-renewal and differentiation into many cell lineages.[3] These experiments set the stage for the identification, characterization, and isolation of stem cells.

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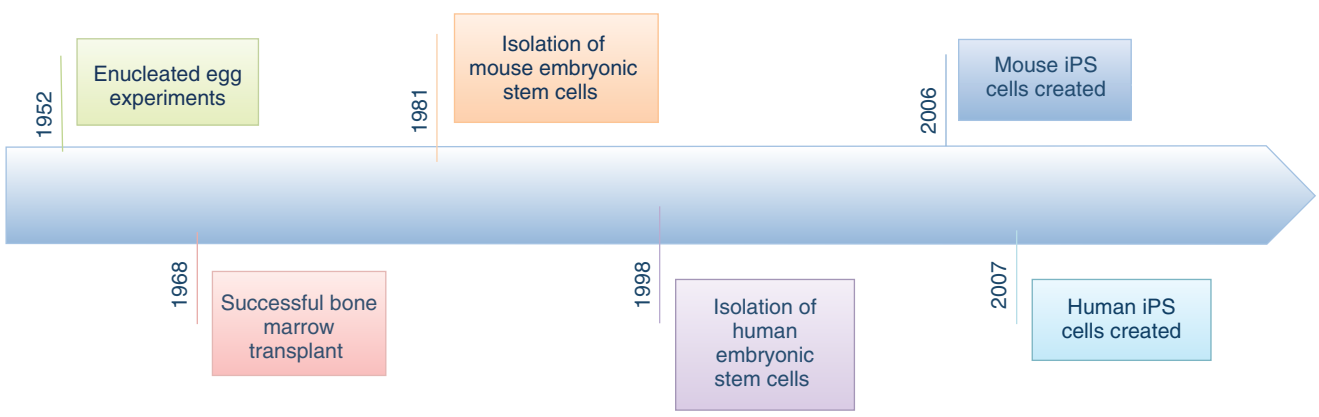


**FIGURE 1.1.** *Abbreviated timeline of stem cell research.*

## Adult Stem Cells

Adult stem cells, or ASCs, were the first type of stem cells to be explored for use in clinical therapy. ASCs are present in many tissues and organs, including bone marrow, skin, muscle, and fat, in which they produce new, healthy cells to replace those that have been damaged (Fig. 1.2).

Hematopoietic stem cells (HSCs) are specialized adult stem cells. The existence of a common HSC was a topic of debate for several decades until definitive evidence was provided by the work of James Till, Ernest McCulloch, and others in the 1960s. Till and McCulloch demonstrated that a single bone marrow cell could give rise to different types of blood cells.[4,5] Pioneering research on bone marrow transplantation by E. Donnall Thomas established that injecting bone marrow cells into the bloodstream could repopulate the bone marrow and produce more blood cells.[6] On the basis of this pivotal demonstration, Dr. Robert A. Good at the University of Minnesota performed the first successful bone marrow transplant in

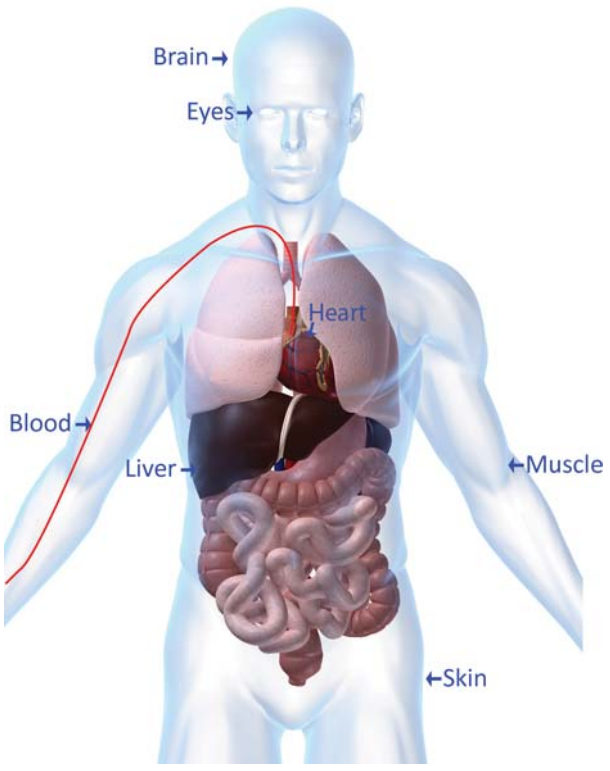


**FIGURE 1.2.** *Known sites of adult stem cells (ASCs).*

1968.[7] Bone marrow transplantation remains a therapy that effectively treats many cancer patients every year.

Unfortunately, ASCs do not have limitless potential, but are currently thought to be restricted to becoming only one or a few specific types of cells. While more types of ASCs are known to exist than previously thought, they have not been found in every organ and tissue in the human body. Additionally, they are difficult to maintain and do not multiply indefinitely in culture.

## Embryonic Stem Cells

While ASCs are classified as "multipotent," meaning they have the potential to become many, but not all cell types, embryonic stem cells (ESCs) are categorized as "pluripotent," meaning they can give rise to all the cells in the body. Four to six days after fertilization of an egg, a human embryo is a blastocyst, a hollow ball of cells, that contains an inner cluster of cells designated the inner cell mass (ICM). When the cells of the ICM are removed from the blastocyst and cultured, they retain pluripotency and can be directed through controlled interventions to become specialized cells (Fig. 1.3a).

Murine embryonic stem cells were first isolated from mice in 1981.[8,9] It was not until 1998 that James Thompson at the University of Wisconsin obtained embryonic

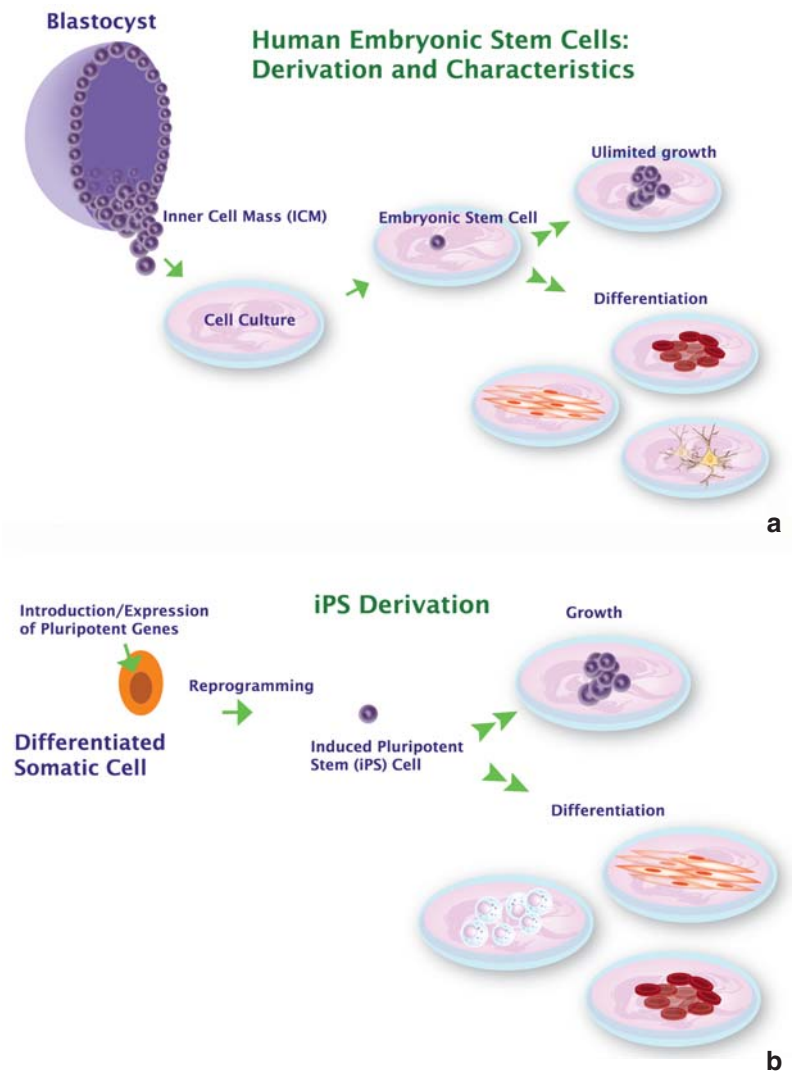


**FIGURE 1.3.** *Derivation of embryonic stem cells and iPS cells.*

stem cells from humans.[10] After extensive testing, Thomson and colleagues verified that they had isolated human stem cells characterized by two important properties; they could multiply indefinitely and they could develop into specialized tissue types. However, the most compelling proof of stem cell pluripotency is their ability to produce an entire organism, which, due to ethical concerns, has only been successfully demonstrated with mouse ES and iPS cells.[11,12]

Most blastocysts used for human stem cell research are donated by couples who have an excess of embryos after completion of an in vitro fertilization (IVF) treatment. The ethical concerns associated with the derivation of human ES cells have been expressed by some groups and has influenced the availability of federal funding for embryonic stem cell investigation. Nevertheless, ES cells have the potential to provide insights into human development as well as offer a wealth of potential therapies.

## iPS Cells

The use of ES cells in research has created emotionally charged bioethical issues that have resulted in loss of US federal funding, therefore inhibiting scientific progress. The development of human iPS cells in 2007 helped to circumvent some of these obstacles. Unlike ES cells, iPS cells will have the potential to overcome compatibility issues when transplanted.

The iPS cells are somatic cells that have been reprogrammed to resemble embryonic stem cells (Fig. 1.3b). Mouse iPS cells were first reported in 2006,[13] and human iPS cells followed soon thereafter in 2007.[14] Both mouse and human iPS cells appear nearly identical to embryonic stem cells in terms of defining criteria: mouse iPS cells express stem cell markers and form tumors containing cells from all three germ layers, which are precursors of all cell types. In addition, mouse iPS cells are able to give rise to an entire mouse when injected into a mouse. Human iPS cells also express stem cell markers and are capable of generating cells characteristic of all three germ layers. Although these cells meet the defining criteria for pluripotent stem cells, more research will need to be done to determine whether iPS cells and embryonic stem cells differ in significant ways.[15]

While there is great hope in the potential of clinical therapies utilizing iPS cells, a number of questions must first be answered. Pluripotency is often reestablished in differentiated cells by expression of several nuclear proteins that are preferentially expressed in ES cells. The use of viruses to introduce genes encoding the pluripotency factors may cause mutations by viral insertion into the genome. Induced expression of the pluripotency factors can result in aberrant control of proliferation. However, recent advances in inducing pluripotency directly with proteins under controlled conditions may eliminate these risks. In the future, in situ programming may provide a window of opportunity for repair and replacement of cells and tissues in order to correct structural or metabolic defects that are associated with cancer, kidney, and neurological diseases (Table 1.1).

**TABLE 1.1** SUMMARY OF TYPES OF STEM CELLS

| *Cells* | *Ethical Concerns* | *Immune Rejection Concerns* | *Cancer Concerns* | *Cell/Tissue Types* | *Availability* |
|---|---|---|---|---|---|
| hESCs | Yes | Yes | Yes | All | Many lines |
| Adult stem cells | No | No | No | Limited | Many (limited types) |
| iPS cells | No | No | Yes | All | Many |

## OUTLOOK OF THE FIELD

There are many expectations and hopes for potential applications of embryonic stem cells and iPS cells. These therapeutic applications include tissue and organ regeneration, the potential for drug screening and toxicity assessment, and interfaces with gene therapy and tissue engineering.

To translate these expectations to reality, there are a number of challenges that the field will need to surmount. While a defining characteristic of both ES and iPS cells is their pluripotency, this same characteristic may result in the formation of tumors. Thus for therapeutic applications, a blueprint of instructions for differentiation that results in pure cultures of differentiated cells will be necessary. In vitro genetic and epigenetic modifications that direct the ES cells to the required cell type may provide solutions. Another challenge to using pluripotent cells in transplantation is the certainty that they will function correctly once transplanted. This is particularly relevant if the transplant is to damaged or diseased organs.

There is still much to be learned about the properties of pluripotent stem cells. The stem cell field is positioned to build on the enormous progress that has been made over the past five decades. It is recognized that a more in depth and systematic investigation is essential to establish an understanding of the maximal potential that stem cells hold for our future. Given advances that are emerging in stem cell biology and technologies, there is confidence that stem cells will provide opportunities to maximize function and enhance quality of life.

## REFERENCES


1. Briggs R, King TJ. Transplantation of living nuclei from blastula cells into enucleated frogs' eggs. *Proc Natl Acad Sci USA*. 1952;38:455–463.
2. Gurdon JB, Elsdale TR, Fischberg M. Sexually mature individuals of *Xenopus laevis* from the transplantation of single somatic nuclei. *Nature*. 1958;182:64–65.
3. Kleinsmith LJ, Pierce GB Jr. Multipotentiality of single embryonal carcinoma cells. *Cancer Res*. 1964;24:1544–1551.
4. Till JE, McCulloch E. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat Res*. 1961;14:213–222.
5. Till JE, McCulloch EA, Siminovitch L. A stochastic model of stem cell proliferation, based on the growth of spleen colony-forming cells. *Proc Natl Acad Sci USA*. 1964;51:29–36.
6. Thomas ED, Lochte HL Jr, Lu WC, et al. Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy. *N Engl J Med*. 1957;257:491–496.
7. Gatti RA, Meuwissen HJ, Allen HD, et al. Immunological reconstitution of sex-linked lymphopenic immunological deficiency. *Lancet*. 1968;2:1366–1369.
8. Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci USA*. 1981;78:7634–7638.
9. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. *Nature*. 1981;292:154–156.
10. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. *Science*. 1998;282:1145–1147.
11. Nagy A, Rossant J, Nagy R, et al. Derivation of completely cell culture-derived mice from early-passage embryonic stem cells. *Proc Natl Acad Sci USA*. 1993;90:8424–8428.
12. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. *Nature*. 2007;448:313–317.
13. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;126:663–676.
14. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007;131:861–872.
15. Yamanaka S. Strategies and new developments in the generation of patient-specific pluripotent stem cells. *Cell Stem Cell*. 2007;1:39–49.

# 2 RESEARCHING AND OBTAINING ESTABLISHED STEM CELL LINES

*Mai X. Luong, Kelly P. Smith, and Gary S. Stein*

Since the original derivation of human embryonic stem cells (hESCs) in 1998,[1] and with the development of new technologies such as cellular reprogramming,[2,3] the stem cell field has expanded rapidly. Currently, it is estimated that over 1000 different hESC lines have been derived worldwide. In addition, reprogramming has made it possible for most labs to develop pluripotent stem cells tailored to their research, such as disease–specific induced pluripotent stem (iPS) cells. This rapid expansion of the stem cell field has necessitated the development of valuable resources to aid the researcher. Registries, which serve as repositories of stem cell information, as well as banks, which are physical repositories for stem cells, have become necessary tools for stem cell research.[4–6]

## RESEARCHING STEM CELL INFORMATION

As research into the properties and therapeutic potential of human pluripotent stem cells accelerates, it becomes more difficult for the researcher to remain current with the available information. Thus there is a need to organize and integrate current knowledge of pluripotent stem cells in a manner that is comprehensive and readily accessible. Registries are databases of information intended to assist researchers by providing extensive and up-to-date information on human pluripotent stem cells. To provide this information, registries must be aware of and constantly adapt to the volume of research findings and the complexity of issues in a rapidly expanding field.

### Technical Questions

There are several key characteristics that an hESC line should possess. These properties include karyotype stability, retention of an undifferentiated state through repeated cell division cycles and prolonged culture, competency for lineage commitment, and the ability for reproducible terminal differentiation into a variety of cell types. However, there are frequent reports of heterogeneity between cell lines and within the same cell lines in different laboratories. Differences between cell lines can be attributed to causes that include embryo quality and stage, and variations in the method and reagents (such as feeder cells) used in the derivation. Genetic variation, medical history of donors, and even the maternal diet can influence the phenotype of cell lines.[7] Characteristics of the same cell line can vary significantly depending on the culture conditions used.[8] All of these variations can influence the ability of the

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ES cells to differentiate. Furthermore, approaches and reagents used to induce differentiation have varying degrees of success. Thus a challenge of hESC research is to obtain comprehensive knowledge of the cell lines and the consequences of disparate technical tools utilized for isolation, maintenance, propagation, and differentiation.

## Intellectual Property Issues

The intellectual property environment in stem cell research, especially for hESCs, is extremely complex. The Wisconsin Alumni Research Foundation (WARF) and James Thomson of the University of Wisconsin were awarded a very broad US patent (#6,200,806) on the isolation of human embryonic stem cells on March 13, 2001. The patent claims are sufficiently broad–based that any use of hESCs for any purpose may fall under the WARF patent.

Currently, WiCell Research Institute Inc. (a subsidiary of WARF) requires a licensing agreement, or Memorandum of Understanding (MOU), acknowledging WARF's patent rights, for the distribution of any human ES cell lines in the United States, regardless of their source or NIH approval status. In addition, any university receiving human ES cells for research is expected to sign a MOU with WiCell. As WARF patents are not recognized outside the United States, US investigators may be at a disadvantage in pursuing commercial applications of hESCs. In addition to the WARF patents, the increased patent protection for stem cells and related technologies in the United States has raised concerns about the emergence of a patent thicket in which overlapping claims block therapeutic applications of hESCs and the pathways to market—both by causing uncertainty about Freedom To Operate (FTO) and by imposing multiple transaction costs.[9]

## hESC Research Guidelines: A Regulatory Maze

The regulatory environment surrounding hESC research is complex in the United States and globally. Various governments around the world and individual states in the United States have their own regulations, which range from permissive to an outright ban on hESC research. In an effort to simplify the complex patchwork of guidelines within the United States and around the world, several groups have produced, or are in the process of developing, guidelines for hESC research that would provide standards for the derivation, procurement, banking, distribution, and applications of human ES cells and create universally accepted documents such as informed consent forms and material transfer agreements (Table 2.1). In addition, groups such as the Interstate Alliance on Stem Cell Research (IASCR, www.iascr.org) are working to facilitate collaborative hESC research across state lines within the Unites States, whereas others, such as the International Society for Stem Cell Research (ISSCR, www.isscr.org), have focused on facilitating collaboration across international borders. It has been suggested that the solution to the lack of cohesion across regulatory frameworks may reside in reciprocal policy agreements. For example, the California Institute for Regenerative Medicine (CIRM, www.cirm.ca.gov) regulations allow funding for hESC research that utilizes cell lines that were derived in the United Kingdom under the Human Fertilization and Embryology Authority license or in accordance with the Canadian Institutes of Health Research Guidelines.[10] CIRM also has a reciprocal agreement with Japan and would consider entering into an agreement with any country that has a body established for active certification of hESC lines. Despite these efforts, the various guidelines shown in Table 2.1 each have their own set of rules regarding provenance of hESC lines, including specific requirements about informed consent and donor reimbursement.

Choosing from among the hundreds of existing hESC lines requires much more than knowledge of their scientific qualities. When obtaining hESC lines, the researcher must consider the guidelines, intellectual property issues, and legislation