Plan for Implementation of Executive Order 13435:
Expanding Approved Stem Cell Lines in Ethically Responsible Ways

Department of Health and Human Services
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I. EXECUTIVE SUMMARY

On June 20, 2007, President George W. Bush issued Executive Order 13435. The Executive Order requires that *"The Secretary of Health and Human Services shall conduct and support research on the isolation, derivation, production, and testing of stem cells that are capable of producing all or almost all of the cell types of the developing body and may result in improved understanding of or treatments for diseases and other adverse health conditions, but are derived without creating a human embryo for research purposes or destroying, discarding, or subjecting to harm a human embryo or fetus."*

The Secretary of the Department of Health and Human Services (HHS) has tasked the National Institutes of Health (NIH) with the responsibility to develop a plan to implement the Executive Order. A summary of the implementation plan is described below.

1. Issue Funding Opportunity Announcements (FOA)

The NIH Stem Cell Task Force will develop several Agency-wide Funding Opportunity Announcements (FOAs) to accelerate research on human pluripotent stem cells (hPSCs) from non-embryonic sources. The FOAs will include a Program Announcement (PA) soliciting research applications proposing research on hPSCs from non-embryonic sources, such as reprogramming somatic cells, deriving cells from amniotic fluid and other sources for developing pluripotent stem cells.

Another FOA will propose Administrative Supplements to add funds to an existing grant. One Supplemental Program will support research on creating hPSCs from non-embryonic sources through the application of technology developed using animal cells. A second Supplemental Program will support research to establish pluripotency of existing human cell lines.

2. Rename the NIH Stem Cell Registry as the Human Pluripotent Stem Cell Registry

The NIH will rename the NIH Human Embryonic Stem Cell Registry as the NIH Human Pluripotent Stem Cell Registry. Consistent with applicable law, the NIH Stem Cell Task Force will develop an NIH definition of "pluripotency" and will use this definition to determine which lines are eligible to be added to the Registry. The Task Force will assess recent advances in stem cell characterization and specify additional criteria as it deems necessary. Scientists interested in having their cell lines included on the Registry can apply to the NIH for consideration.

3. Consider Alternative Sources of Pluripotent Stem Cells

NIH will explore research on alternative sources of pluripotent stem cells, including specifically those techniques outlined in a 2005 white paper by the President's Council on Bioethics (PCOB) entitled "Alternative Sources of Human Pluripotent Stem Cells." This report discusses four potential sources of pluripotent stem cells: "dead" embryos, altered nuclear transfer (ANT), single cell biopsy, and cellular reprogramming. In addition to these methods, other potential sources of pluripotent stem cells should be considered that are consistent with the Executive Order and applicable law and policy.

4. Undertake Comprehensive Portfolio Analysis

NIH will conduct a comprehensive review of its research portfolio to determine if research is currently being conducted on any of the techniques mentioned above. This portfolio analysis will assist NIH in determining which alternative methods represent unmet research opportunities. NIH will then work to address these unmet research opportunities by including these methods in a PA.

5. Convene State-of-the-Science Workshop

NIH will convene a workshop to evaluate the state of the science of the various ways to derive hPSC lines, identify knowledge gaps and determine what specific techniques/methods may require additional basic or animal research to ensure that any research involving human cells using these techniques is consistent with the standards established under the Executive Order and applicable law and policy. The outcome of the workshop will inform the NIH as to needs for additional FOAs.

6. Hold Human Pluripotent Stem Cell (hPSC) Research Symposium to Prioritize Research with the Greatest Potential for Clinical Benefit

The NIH will convene a symposium on the current state of basic and clinical pluripotent stem cell biology to help NIH prioritize research with greatest potential for clinical benefit. This symposium will enable NIH to determine which areas of high priority research need to be emphasized and then to develop additional FOAs to increase research in these areas.

II. INTRODUCTION

Overview

This plan responds to the President's Executive Order 13435 of June 20, 2007, that *"The Secretary of Health and Human Services shall conduct and support research on the isolation, derivation, production, and testing of stem cells that are capable of producing all or almost all of the cell types of the developing body and may result in improved understanding of or treatments for diseases and other adverse health conditions, but are derived without creating a human embryo for research purposes or destroying, discarding, or subjecting to harm a human embryo or fetus."*

On August 9, 2001, President George W. Bush announced his decision (President's Policy) to allow federal funds to be used for human embryonic stem cell (hESC) research in ways that would not encourage the further destruction of human embryos. Since the President's Policy was announced, the NIH has funded human embryonic stem cell (hESC) research and advanced research on all forms of stem cells, including those from non-embryonic sources in a way that is ambitious, ethical, and effective.

Stem cells have been recognized as a tool for advancing our knowledge about cell specialization, and its great potential to be medically valuable. However, using established methods, hESCs cannot be obtained without destroying human embryos. Recently, technical innovation in stem cell derivation and proliferation suggests that there may be new avenues for scientific progress in this arena.

In 2005, the PCOB published a white paper on "Alternative Sources of Human Pluripotent Stem Cells." The potential sources described in the 2005 PCOB report include so-called "dead" embryos; ANT; single cell embryo biopsy; and reprogramming, or dedifferentiation of somatic cells. Although it is not described in the PCOB report, another potential source of pluripotent stem cells is human amniotic fluid. Recent publications that have described potential sources of pluripotent stem cells and the relevant original scientific journal articles are discussed in detail in starting on page 9.

It is important to note that, while many of these alternative approaches show great promise, all of them remain as yet unproven as a means to derive hPSCs. To this end, NIH is supporting research on alternative methods to develop pluripotent stem cells, primarily using animal models at first. Of particular interest, recent studies, described in more detail below, show that adult mouse cells can be reprogrammed to behave like embryonic stem cells. As they are applied to human research, all new approaches must be carefully considered by scientists, ethicists, and regulators on a case-by-case basis, as further data become available. These approaches must also be analyzed in light of the President's Policy and the appropriations provision known as the Dickey Amendment or Human Embryo Research Ban (HERB). For FY 2006 and FY 2007, the HERB language

can be found in Section 509 of the Labor, HHS, and Education and Related Agencies Appropriations Act, 2006: H.R. 3010, H. Report 109-143).

Background

What are Stem Cells?

Stem cells are unspecialized cells that renew themselves for long periods through cell division. In addition, stem cells can be induced, under certain physiologic or experimental conditions, to become cells with special functions, such as a muscle cell, a red blood cell, or a brain cell.

The Potential of Stem Cells

Studying stem cells will help us understand how they differentiate into the array of specialized cells that make us what we are. Some of the most serious medical conditions, such as cancer and birth defects, are due to problems that occur somewhere in this differentiation process. A better understanding of normal cell development will allow us to understand and perhaps correct the errors that cause these medical conditions.

Another potential application of stem cells is making cells and tissues for medical therapies. Today, donated organs and tissues are often used to replace those that are diseased or destroyed. Unfortunately, the number of people needing a transplant far exceeds the number of organs available for transplantation. Pluripotent stem cells offer the possibility of a renewable source of replacement cells and tissues to treat a myriad of diseases, conditions, and disabilities including Parkinson's disease, spinal cord injury, stroke, burns, heart disease, diabetes, and osteo- and rheumatoid arthritis, to name a few.

Scientists have only been able to perform experiments with hESC since 1998, when a group led by Dr. James Thomson at the University of Wisconsin first developed a technique to isolate and grow the cells. Federal funds to support hESC research were made available on August 9, 2001, when President Bush announced his Policy. Because many academic researchers rely on Federal funds to support their laboratories, they are just beginning to learn how to grow and use the cells. Thus, although hESC are thought to offer potential cures and therapies for many devastating diseases, research using them is still in its early stages.

Adult stem cells, such as blood-forming stem cells in bone marrow (called hematopoietic stem cells, or HSCs), are currently the only type of stem cell commonly used to treat human diseases. Doctors have been transferring HSCs in bone marrow transplants for over 40 years. More advanced techniques of collecting or "harvesting" HSCs are now used to treat leukemia, lymphoma, and several inherited blood disorders. Scientists are

exploring additional clinical potentials of adult stem cells by determining whether other diseases and disorders can be treated with cell-based therapies.

In August 2002, the NIH Director, Dr. Elias Zerhouni, established the NIH Stem Cell Task Force to monitor the state of this rapidly evolving area of science. The purpose of the Task Force is to enable and accelerate the pace of stem cell research by identifying rate limiting resources and developing initiatives to overcome these barriers to progress.

Over the past five years, under the leadership of the Task Force, the NIH has supported a wide array of scientific programs designed to foster research on hESCs lines, and is actively working to fund research in this blossoming field of investigation. For example, the Task Force has stimulated NIH-supported research by initiating Infrastructure grants to scale-up and characterize stem cells eligible for Federal funding, developed training courses to teach stem cell culture techniques, established a National Stem Cell Bank to make hESC lines readily available, and encouraged new investigator-initiated research through various means.

Working with the NIH Stem Cell Task Force is the NIH Stem Cell Implementation Committee. The Implementation Committee consists of scientific program officials from the NIH Institutes and Centers (ICs) which support and conduct stem cell research. While members of the Implementation Committee have the responsibility to oversee the stem cell research program within their respective IC, and the Task Force routinely works with the Implementation Committee on trans-NIH stem cell issues, such as developing new research initiatives that involve multiple ICs.

III. IMPLEMENTATION PLAN FOR EXECUTIVE ORDER 13435

1. Issue Funding Opportunity Announcements

NIH already receives applications from investigators proposing experiments to address the development, characteristics, and use of hPSCs. NIH will continue to review them and fund the ones that represent the most promising avenues. To accelerate research on hPSCs from non-embryonic sources, the NIH Stem Cell Task Force will develop several Agency-wide FOAs. A FOA is a notice of a Federal grant funding opportunity. A FOA's synopsis page is linked to an NIH Guide announcement, which provides investigators with opportunity-specific information and instructions to apply for Federal funding.

The NIH Stem Cell Task Force will develop several FOAs, including a PA, as well as several Notices for Supplements (NOT) to existing grants, each of which is described in more detail below.

Program Announcement (PA)

A PA is an NIH announcement requesting grant applications in stated scientific areas. Examples of NIH-issued PAs can be found on the NIH Guide for Grants and Contracts website. The NIH Stem Cell Task Force will develop a PA requesting the submission of grant applications proposing research on hPSCs derived from non-embryonic sources, for example, from somatic cells or cells found in amniotic fluid. It is advantageous to use a PA, rather than a Request for Applications (RFA), to solicit such research applications for the following reasons. A PA remains active for several years. In addition, investigators may submit their applications in response to the PA on any of the three application receipt dates per year during which NIH accepts investigator-initiated grant applications. Thus, a PA provides a significant advantage over an RFA in which there is only one chance to respond to the request. Therefore, the PA allows investigators to come in with applications addressing the subject of the PA when their research proposal is ready to be submitted. This greatly widens the opportunity to submit an application in response to the PA and should result in more applications over time. Also, as the science progresses over time, applications will incorporate advances in the field of pluripotent stem cell research.

The PA will accept applications using multiple NIH grant mechanisms, which also will offer more flexibility and maximize the opportunities for investigators working in this important area of research. The specific grant mechanisms that will be used include the R01, the R03, and the R21.

a. The R01 Research Project Grant is used to support a discrete, specified, circumscribed research project and is NIH's most commonly used grant program. There is no dollar limit unless specified in the FOA and it is generally awarded for three to five (3-5) years.

b. The R03 is the NIH Small Grant Program. R03s provide limited funding for a short period of time to support a variety of types of projects including pilot or feasibility studies; collection of preliminary data; secondary analysis of existing data; small, self-contained research projects; and development of new research technology. R03s are generally limited to two (2) years of funding with direct costs of up to $50,000 per year.

c. The R21 is the NIH Exploratory/Developmental Research Grant Award and encourages new, exploratory, and developmental research projects by providing support for the early stages of project development and/or pilot and feasibility studies. R21s are generally limited to two (2) years of funding and the combined budget for direct costs of the project usually may not exceed

$275,000. Because the R21 encourages exploratory research, relatively little preliminary data are required.

Notices for Administrative Supplements (NOT)

Supplements add funds to an existing grant to pay for items within the scope of an existing grant award that were unforeseen when the initial grant application was submitted. Examples of NIH-issued Supplements can be found on the NIH Guide for Grants and Contracts. Supplements are particularly advantageous because they provide a mechanism to stimulate research in a specific area very rapidly. The supplemental funds are rapidly transferred to researchers already working in the area of interest to augment certain areas of their work that are of particular interest to NIH. Two notices for Supplements will be published in the NIH Guide for Grants and Contracts.

a. The first Supplemental Program will fund research on hPSCs derived from non-embryonic sources, such as through somatic cell reprogramming. For example, NIH-supported researchers with grants to study somatic cell reprogramming in mouse cells will be eligible for supplemental funding to use what they have learned in their animal experiments and apply it to research on reprogramming somatic human cells.

b. The second Supplemental Program will fund research to test the pluripotency of existing human cell lines. For example, researchers with grants that utilize cell lines that appear capable of differentiating into multiple cell types will be eligible for supplemental funding to perform additional experiments on these cells to determine if the cells are indeed pluripotent and therefore eligible for inclusion on the NIH Pluripotent Stem Cell Registry (details discussed below).

2. Rename the NIH Stem Cell Registry as the Human Pluripotent Stem Cell Registry

Executive Order 13435, "Expanding Approved Stem Cell Lines in Ethically Responsible Ways," requires the NIH to rename the NIH Human Embryonic Stem Cell Registry as the "NIH Human Pluripotent Stem Cell Registry." At present, there is no standardized definition of the term "pluripotency." The NIH will call upon the expertise of the NIH Stem Cell Task Force to develop an NIH definition of "pluripotency," consistent with applicable law. The Task Force will then use this definition to determine additional lines for listing on the Registry. The Task Force will assess recent advances in pluripotent stem cell characterization and specify additional criteria as it deems necessary. The Task

Force will determine if the lines proposed for inclusion in the Registry are consistent with the policies and principles of the Executive Order.

Any scientist interested in submitting cell lines for inclusion on the NIH Human Pluripotent Stem Cell Registry must apply to the NIH for consideration. The application process will include submission of the form "Application for Inclusion of Human Pluripotent Stem Cells on the NIH Human Pluripotent Stem Cell Registry" (*in development),* and documentation of the performance of the cells in a series of standard scientific pluripotency assays. The assays will be chosen for their ability to determine whether the cells satisfy all of the characteristics included in the NIH definition of pluripotency.

Applications for inclusion on the NIH Human Pluripotent Stem Cell Registry (the Registry) will be reviewed by a subcommittee of the NIH Stem Cell Task Force, known as the NIH Human Pluripotent Stem Cell Registry Committee (the Registry Committee). This committee shall consist of members of the NIH Stem Cell Task Force, and shall make final recommendations regarding proposed cell lines. The Registry Committee shall then present the Task Force with a list of those cell lines it deems eligible for inclusion based on the criteria for pluripotency and a determination that the creation of the cells is consistent with the Dickey Amendment, the President's Policy and the Executive Order. Applicants will be notified in writing whether or not their lines will be included on the Registry. The NIH Stem Cell Task Force will then add these new cell lines to the NIH website for the Human Pluripotent Stem Cell Registry.

NIH will accept grant applications that propose research using cells listed on the Registry. Grant applications that propose research on cells that are derived from human embryos or alternative sources, but are not listed on the Registry, will be required to provide evidence in the grant application that they were derived consistent with the policies and principles of the Executive Order; the President's Policy; and applicable law, including the Dickey Amendment.

3. Consider Alternative Sources of Pluripotent Stem Cells

Executive Order 13435, "Expanding Approved Stem Cell Lines in Ethically Responsible Ways," Section 1, Subsection b, Part iii specifically directs NIH to take "into account techniques outlined by the PCOB, and any other appropriate techniques and research, provided they clearly meet the standard set forth in subsection (a) of this section." This portion of the NIH Executive Order Implementation Plan considers Alternative Sources of Pluripotent Stem Cells.

Available Alternative Sources of Pluripotent Stem Cells

The PCOB white paper entitled "Alternative Sources of Pluripotent Stem Cells" was released in May 2005. The paper discusses four potential alternative sources of pluripotent stem cells: "dead" embryos; ANT; single cell embryo biopsy; and reprogramming, or dedifferentiation, of somatic cells. In addition to these sources, new sources of pluripotent stem cells are also being discovered, as discussed in the section entitled "Other Potential Sources of Pluripotent Stem Cells" on page 15.

Pluripotent Stem Cells from Dead Embryos

Scientists proposing this method noted that, during the human in vitro fertilization (IVF) process, there are numerous embryos that stop dividing, and are therefore judged to be unsuitable for implantation. They argue that these non-dividing entities are "dead," and they propose that harvesting cells from these embryos for the purpose of creating a hESC line is no different than organ donation by a person judged to be "brain dead." They argue that this approach is morally acceptable.

In a privately funded study published in 2006, these scientists evaluated the physical characteristics of human embryos created for IVF, but were not used for reproductive purposes because they were considered to be "nonviable," i.e., not capable of living or developing successfully. The scientists observed that many of the embryos classified as nonviable had fewer cells than normal and failed to compact into a morula (in human development, a 4-day old, preimplanted embryo) or a blastocyst (in human development, a 5-day old, preimplanted embryo). They propose that nonviable embryos with these features of arrested development at 5 days post-fertilization be considered "organismically dead". This would allow scientists to harvest cells from such "organismically dead" human embryos in experimental efforts to generate human embryonic stem cell lines. (*Regen Med*. 1: 367-371, 2006).

In 2007, scientists funded by the United Kingdom and other private sources successfully generated human embryonic stem cell (hESC) lines from in vitro fertilization (IVF) embryos that stopped developing naturally. The human stem cells created using this technique behaved like pluripotent stem cells, including making proteins critical for so-called "stemness" and producing cells from all three germ layers. (*Stem Cells*. 24: 2669–2676, 2006).

To be useful for basic or clinical research, stem cells derived from so-called "dead" embryos must be carefully monitored for karyotypic

(genetic) abnormalities or other defects, which may have been the underlying cause of the embryo's lack of development. For this research to be considered for Federal funding pursuant to the Dickey Amendment, NIH will establish clear criteria that will determine when a non-dividing embryo is organismically dead.

Altered Nuclear Transfer (ANT) -- Making Pluripotent Stem Cells from Biological Artifacts

ANT is a general concept that its proponents suggest could take a number of specific forms. Proponents of this method assert, for instance, that it may be possible to do the following: (1) genetically modify a somatic cell in culture, for instance, the cell might be engineered to lack a gene or genes crucial for cell-to-cell signaling or the integrated organization essential for normal embryogenesis; (2) use this genetically modified somatic cell as the source of a nucleus and genome for somatic cell nuclear transfer (SCNT) into a human oocyte. This method is referred to as ANT; (3) allow the resulting entity to develop to a point when it may yield embryonic-like stem cells; and (4) after extraction, attempt to generate a hESC or hESC-like line from these cells.

One version of the idea proposes that scientists turn off a gene needed for implantation in the uterus (*Cdx2*) in the individual's cell nucleus before it is transferred into the donor egg. NIH-supported scientists recently reported proof of principle tests that ANT works in mice. Mouse ANT entities whose *Cdx2* gene is switched off are unable to implant in the uterus and do not survive to birth. Scientists used ANT to create viable stem cell lines capable of producing almost all cell types. The scientists point out that this technique must still be tested with monkey and human donor nuclei, and the manipulation needed to control *Cdx2* expression introduces another logistical hurdle that may complicate ANT's use to derive embryonic stem cells. (*Nature*. 439: 212–215, 2006).

The availability of Federal funding for research involving any of the specific forms of ANT and ANT-derived lines will depend upon whether or not the genetically modified entity created using this procedure is determined to be a human embryo for purposes of the Dickey Amendment, the President's Policy, and the Executive Order.

Single Cell Embryo Biopsy

This technique involves the creation of an embryonic stem cell line by using a blastomere (a single cell) from an embryo and builds upon the

techniques used in pre-implantation genetic diagnosis (PGD). In PGD, a single blastomere is removed from an 8-cell stage embryo (approximately Day 3 post fertilization) for genetic analysis. The seven cells that remain constitute an embryo that can be used for reproductive purposes via the standard IVF procedure. The proponents of single cell embryo biopsy suggest that the success of PGD in clinical practice represents proof of principle that removal of a single cell does not damage the remaining embryo. However, in a recent study, scientists observed that PGD significantly reduced the rate of pregnancies and live births after IVF in women of advanced maternal age. (*N Engl J Med*. 357: 9-17, 2007).

The proposal of single cell embryo biopsy for the creation of a hESC line presumes that an additional cell, or several cells, might be removed from an embryo at the same time the embryo is undergoing PGD. Proponents of the technique further claim that if one limits this approach to embryos undergoing PGD, one is: 1) not compromising any embryos that are not already being compromised for PGD; and 2) assuring the embryos being used were created only for reproductive purposes.

Studies using mouse embryos suggest that embryonic stem cell lines can be obtained from single cell biopsy and that the biopsy does not cause significant harm. In early 2006, privately funded scientists removed single cells from early mouse embryos. They used them to establish mouse embryonic stem cell lines. This research was the first to demonstrate that single cell embryo biopsy can be used successfully to generate stem cell lines. Of note, the remaining cells of the embryo were implanted in surrogate mouse wombs and approximately half developed into seemingly normal mouse pups. In the control group of non-biopsied embryos, about half also developed to birth as normal pups. In 2007, the same scientists used this method to establish human embryonic stem cell lines from single cells biopsied from human embryos. Although single cell embryo biopsy proposes to avoid embryo destruction, scientists do not yet know how much risk the procedure might confer to an otherwise healthy human embryo.

A critical and unanswered question relevant to this approach is whether the single cell removed for the purposes of deriving a line is in itself totipotent and capable of developing into a fetus. If this is the case, then its destruction to create a cell line would be no different from the destruction of the embryo from which it was extracted. (*Nature*. 439: 216–219, 2006 and *Nature*. 444: 481-485, 2006). Consequently, any such research would have to be analyzed in light of the Dickey Amendment, the

President's Policy, and this Executive Order before NIH could fund research using stem cells derived from human embryos.

Pluripotent Stem Cells by Reprogramming Somatic Cells

This proposed method involves reprogramming human somatic cells, perhaps with the aid of special cytoplasmic factors obtained from oocytes (or from pluripotent embryonic stem cells), so as to "de-differentiate" them into pluripotent stem cells. Crucial to the success of this approach is discovering a way to reverse cell differentiation back to pluripotency, but not further back to totipotency, since a totipotent cell is considered an embryo for purposes of the Dickey Amendment. Scientists are pursuing numerous avenues to reach this goal.

In 2005, privately funded scientists fused cultured adult human skin cells with hESCs. The resulting "hybrid" cells had many characteristics of hESCs—they grew and divided in a similar manner and manufactured proteins that are typically made in hESCs. Some as-yet unknown factor(s) within the hESCs enabled them to "reprogram" the adult skin cell nucleus to behave as hESCs. There are still significant technical barriers that must be overcome before these cells can be used to treat individuals. Because the fused cells are tetraploid (they contain four copies of the cellular DNA rather than the normal two copies), scientists must develop a method to remove the extra DNA without eliminating their hESC-like properties. If this hurdle can be overcome, this technique could allow scientists to create patient-specific stem cells without using human eggs. At present, this new approach to creating stem cells is a useful model system for studying how stem cells "reprogram" adult cells to have properties of pluripotent cells. (*Science*. 309: 1369–1373, 2005).

In 2006, German scientists succeeded in coaxing adult mouse stem cells that normally produce sperm (spermatogonial stem cells, or SSCs) to behave like embryonic stem cells (ESCs). They accomplished this switch of fate by finding the elusive SSCs in mouse testicles and growing them in the laboratory under standard ESC culture conditions. Under those conditions, the cells made several proteins characteristic of ESCs. The scientists subjected the cells to critical tests for pluripotency, and their results suggest that the cells can become any type of cell in the body. As a result, the scientists named them multipotent adult germline stem cells (maGSCs). If scientists can find similar cells in human testicles, the cells could provide a new source of patient-specific stem cells, and could also provide more pluripotent cell lines for research, if the extraction of such

cells for research purposes were deemed ethically acceptable by an institutional review board. (*Nature*. 440: 1199-1203, 2006).

Privately funded scientists in the United Kingdom reported in 2006 that the reprogramming process in mice is more efficient when they engineer the stem cells to over-express Nanog, a gene important for maintaining stem cells' self-renewing properties. The scientists reported a 200-fold increase in the efficiency of the process when mouse embryonic stem cells that over-expressed Nanog were fused with stem cells from mouse brain. However, the fused cells are tetraploid, meaning that they contain four copies of the cellular DNA rather than the normal two copies. This study demonstrates that Nanog can play an important role in reprogramming the mouse brain cells to a state of pluripotency. If these results can be repeated with human cells, they would represent a first step toward learning how to reprogram adult cells to behave as stem cells and directing them to become specific cell types for use in treating human beings. Scientists must still learn how to remove the extra set of cellular DNA without removing the stem-cell like characteristics. (*Nature*. 441: 997-1001, 2006)

In 2006, Japanese scientists reported that they could use a virus to introduce four important stem cell factors into adult mouse cells and reprogram them to behave like embryonic stem (ES) cells. They called the reprogrammed cells iPS, for induced pluripotent stem cells. However, iPS produced using the original technique cannot do everything that ES cells can do. Notably, the original iPS cells were unable to make sperm and egg cells when injected into an early mouse blastocyst, and they do not make some changes to their DNA that help silence genes. As of 2007, the same scientists have modified their original technique, and they report that they can select for iPS that can make sperm and eggs. Their report is accompanied by another from an NIH-supported laboratory which successfully reproduced the Japanese group's results and added a clonal selection step. Using this protocol, the NIH-supported scientists determined that iPS DNA is modified in a manner similar to ES cells, and important stem cell genes are expressed at similar levels. They also demonstrated that iPS injected into an early mouse blastocyst can produce all cell types within the developing embryo, and such embryos can complete gestation and are born alive.

While these research advances are very exciting, the reprogramming has only been accomplished using mouse cells. It is unclear whether the same or other techniques can be developed to reprogram cells of adult humans. If this can be accomplished, scientists should be able to develop stem cell

lines from individuals who suffer from genetic diseases, such as Huntington's disease, spinal muscular atrophy, muscular dystrophy, and thalessemia. Such lines would be invaluable research tools for understanding specific diseases and testing potential drugs to treat them. A second use of reprogrammed cells would be to repair damaged tissues in the human body. The Japanese scientists found that the virus used to introduce the stem cell factors sometimes caused cancers in the mice. This represents a significant obstacle that must be overcome before the technique can lead to useful treatments for humans that involve cell transplantation. (*Nature*. 448: 318-324, 2007 and *Nature*. 448: 313-317, 2007).

Other Potential Sources of Pluripotent Stem Cells

In addition to those methods addressed by the 2005 PCOB report, other promising avenues are also being explored. One example is human stem cells derived from amniotic fluid.

Amniotic fluid surrounding the developing fetus contains cells shed by the fetus and is regularly collected from pregnant women during amniocentesis, a procedure woman can undergo to detect chromosomal abnormalities early in pregnancy. Scientists have previously reported that some of these cells can differentiate into fat, muscle, bone, and nerve cells. Now, privately funded scientists have generated non-embryonic stem cell lines from cells found in both human and rat amniotic fluid. They named the cells amniotic fluid-derived stem cells (AFS). The cells are self-renewing and maintain the normal number of chromosomes after a long time in culture. Tests demonstrate that AFS can produce cells that originate from each of the three embryonic germ layers. However, undifferentiated AFS did not make all of the proteins that characterize embryonic stem cells, and they were not capable of forming a teratoma. The scientists developed in vitro conditions that enabled them to produce nerve cells, liver cells, and bone-forming cells from AFS. AFS-derived human nerve cells could make proteins typical of specialized nerve cells and were able to integrate into a mouse brain and survive for at least two months. Cultured AFS-derived human liver cells secreted urea and made proteins characteristic of normal human liver cells. Cultured AFS-derived human bone cells made proteins expected of human bone cells and formed bone in mice when seeded onto 3-D scaffolds and implanted under the mouse's skin. Although scientists do not yet know how many different cell types AFS are capable of generating, AFS may one day allow scientists to establish a bank of cells for transplantation into human beings. (*Nature Biotechnol*. 25: 100–106, 2007).

4. Undertake Comprehensive Portfolio Review

The NIH will undertake a comprehensive review of its grant portfolio to determine what research NIH is currently supporting that utilizes any of the aforementioned techniques. To conduct the review, NIH will ask members of the NIH Stem Cell Implementation Committee to review their IC's portfolios. Implementation Committee members will then forward a list of any identified projects to the administrative office of the NIH Stem Cell Task Force which will compile a master list. This portfolio analysis will assist NIH in determining which alternative methods are of interest to individual ICs, and which methods represent unmet research opportunities. NIH will then work to address these unmet research opportunities by including these methods in its forthcoming PA, as described previously.

Information collected from the portfolio review will be reviewed by the NIH Stem Cell Task Force, who will assess the current science, identify gap areas in the research, and determine areas that can result in additional research opportunities. This assessment will also be shared with the participants of the stem cell workshop (see below) to provide them with the status of NIH's current research portfolio on alternative methods to derive pluripotent stem cells.

To be eligible for federal funding, the research techniques mentioned here must be consistent with the appropriations provision known as the Dickey Amendment, the President's Policy, and the Executive Order. In the event any of the techniques are deemed ineligible for federal funding, the NIH will only be able to fund such techniques in animal models. In other cases, progress in a particular technique may not yet be advanced enough to warrant its application to human cells. In this regard, the NIH will be informed by discussion at the alternative methods workshop.

5. Convene State-of-the-Science Workshop

The NIH will convene a State-of-the-Science workshop to assess the various ways to derive hPSC lines, identify knowledge gaps, and determine what specific techniques/methods may require additional basic or animal research to ensure that any research involving human cells using these techniques is consistent with the standards established under Executive Order 13435; the President's Policy; and applicable law, including the Dickey Amendment

The workshop will evaluate the scientific information available on the derivation techniques of hPSCs including clinical, legal, and ethical considerations to determine directions for future research. The workshop will consist of a panel of clinical and basic scientists with the relevant expertise from academic medical centers, industry, and appropriate advocacy organizations. The panel should represent various sectors of

professional and community life, including each of the following four general categories: research investigators in the field--that is, scientists who are active in the area under consideration; health professionals who use the technology, including practicing physicians, dentists, psychologists, nurses, or other health care providers; methodologists, such as epidemiologists; and biostatisticians. Public representatives on the panel may include ethicists, lawyers, theologians, economists, public interest group or voluntary health association representatives, consumers, and individuals. The panel chairperson will be a knowledgeable and prestigious figure in the field of biomedical research under consideration but should not be identified with an advocacy position on the conference topic or with research that might be presented to answer conference questions. A broad range of expertise is critical to the panel's ability to assess the scientific material to be evaluated and to insure the credibility of the report.

The State-of-the-Science workshop will give balanced, objective, and knowledgeable attention to the topic. The panel will listen to the scientific data presented by invited experts and comments from the general public. Invited experts will present data to the panel in public sessions, followed by rigorous and lengthy discussion. Thus, the workshop will provide a "snapshot in time" of the state of knowledge on the conference topic. The creative work of the panel will be to synthesize this information, along with potentially conflicting interpretations of the data, into clear and accurate answers to the questions posed to the panel. The entire panel, working in subgroups, will draft a summary of the discussions during executive sessions. The panel should attempt to reach consensus on each question based on the scientific evidence presented. The summary may reflect uncertainties, options, or minority viewpoints.

Speakers will be selected for their scientific expertise and may include clinical investigators and basic scientists as well as general authorities in the field. Where differences of scientific opinion exist, care should be exercised to include the presentation of opposing data and interpretations. Speakers are asked to confine their presentations to the scientific topic that they have agreed to address and to be certain to present all relevant data and information. Speakers are expected to provide abstracts of their presentations.

The announcement of the meeting may be disseminated to all interested groups in a variety of media (for example, brochures, the Internet, professional journals, lay newsletters).

Based on the information presented at the workshop and the statement developed by the panel, the NIH Stem Cell Task Force will determine which derivation techniques would benefit from additional research, and will require additional basic or animal research to ensure that any research involving human cells using these techniques is consistent with the standards established under the Executive Order, the President's Policy, and applicable law, including the Dickey Amendment. The Task Force will work with the

NIH Stem Cell Implementation Committee to develop ideas for research initiatives, such as Requests for Applications, PAs, etc., that will solicit specific research in this area.

6. Hold Human Pluripotent Stem Cell (hPSC) Research Symposium to Prioritize Research with the Greatest Potential for Clinical Benefit

The NIH will organize a Symposium on the current state of basic and clinical pluripotent stem cell biology to help NIH "prioritize research with the greatest potential for clinical benefit" as required by the Executive Order. NIH held a similar symposium on June 12, 2003, entitled "NIH Research: Recent Progress and Future Promise of Human Embryonic Stem Cells".

A symposium planning subcommittee will be formed from members of the NIH Stem Cell Implementation Committee and members of the NIH Stem Cell Task Force to oversee the logistics of the Symposium. Speakers will be selected for their scientific expertise in the area of pluripotent stem cell biology and will include clinical investigators and basic scientists as well as general authorities in the field. Where differences of scientific opinion exist, care will be exercised to include the presentation of opposing data and interpretations. Scientists conducting research on pluripotent human stem cells will be specifically invited to participate in the symposium. The NIH scientific program officers from each IC supporting grants in this area will be asked to contact the grantees about making oral presentations. The topics for the panel discussion will be centered on the current state of basic and clinical pluripotent stem cell biology and pluripotent stem cell research with the greatest potential for clinical benefit.

The morning session of the event will include a plenary session with scientists who are conducting research on hPSCs. The conference plenary session will be moderated by a highly respected member of the scientific community who will ensure that speakers adhere to time limits, allow ample opportunity for scheduled discussion, and invite questions and comments from audience members. The plenary session will be conducted as a panel of scientists speaking on a particular topic or as individual presenters. The afternoon session will include individual breakout sessions/workshops that will help NIH prioritize research on hPSCs with the greatest potential for clinical benefit. The presenters for the workshops may be NIH staff as well as grantees and other relevant experts in the field.

A member of the symposium planning subcommittee will be responsible for summarizing the findings from the symposium. The Stem Cell Task Force will then clearly and accurately synthesize this information, along with any conflicting interpretations of the data. The summary information may reflect uncertainties, options, or minority viewpoints.

Based on the information learned through the symposium and the suggestions offered by experts in the field of pluripotent stem cell biology, the NIH Stem Cell Task Force will review the list of prioritized research developed by the participants at the symposium and determine the best means to encourage this research. The Task Force will work with the NIH Stem Cell Implementation Committee to develop initiatives to encourage further research in pluripotent stem cell biology with the greatest potential for clinical benefit. NIH will host additional symposiums in the future to assess the progress and future promise of pluripotent stem cells. These symposiums will inform NIH and the research community about the further potentials of stem cells.

IV. TIMELINE FOR IMPLEMENTATION OF EXECUTIVE ORDER 13435

Issue Funding Opportunity Announcements (Fall 2007-Summer 2009)

A. Publish Program Announcement (PA)

- IC program official achieves concept clearance
- Concept clearance is approved by IC's advisory council
- IC program official begins developing draft PA and solicits collaboration from other ICs
- PA gets published in Early Notification System
- PA gets published in NIH Guide for Grants and Contracts
- NIH receives grant applications
- Applications get assigned and referred to study section for review
- Study section reviews applications
- Scored applications presented to IC's advisory council
- Notice of Grant Award developed for applications approved for funding released August 2008
- NIH makes grant award to institution with earliest anticipated grant project start date at September 2008
- PA will be re-issued and have multiple receipt dates

B. Publish Notices for Administrative Supplements (NOT)

- Participating IC program official achieves concept clearance
- Concept clearance is approved by participating IC's advisory council
- Participating IC program official begins developing draft Notice
- Notice(s) gets published in NIH Guide for Grants and Contracts
- PI submits written request to the appropriate IC program official
- Requests reviewed by participating IC
- NIH makes grant award to institution with earliest anticipated grant project start date at April 2008

Rename the NIH Stem Cell Registry as the Human Pluripotent Stem Cell (hPSC) Registry (Late Fall 2007-Winter 2009)

- NIH Stem Cell Task Force develops definition of "pluripotency"
- NIH Stem Cell Task Force forms Registry Committee
- NIH Registry Committee determines NIH definition of "dead embryo," and whether ANT, single cell embryo biopsy, other alternative methods

violate either the President's Policy of August 9, 2001, the Executive Order #13435, or the Dickey Amendment
- Registry Committee reviews applications to determine cell line eligibility; review may require interactions with applicants to verify information or request further data
- Registry Committee presents the NIH Stem Cell Task Force with a list of those cell lines it deems eligible for inclusion on the NIH Human Pluripotent Stem Cell Registry
- If NIH Stem Cell Task Force agrees with eligibility list, Registry Committee sends a letter to each applicant, informing her/him of their decision; those not selected will also be notified
- NIH will standardize the data collected regarding eligible cell lines
- Cells deemed eligible will be added to the NIH Human Pluripotent Stem Cell (hPSC) Registry

Undertake Comprehensive Portfolio Review (Fall 2007)

- NIH Stem Cell Task Force administrative office drafts an email to request that each IC's Stem Cell Implementation Committee member work with her/his budget officer to identify projects that include work with alternative methods for deriving pluripotent stem cells; email must also be reviewed and approved by NIH's Office of Budget
- NIH Stem Cell Task Force administrative office and NIH Office of Budget sends email request
- NIH Stem Cell Implementation Committee members review their respective IC's portfolios for research on alternative methods; projects (with funding in whole dollars) reported to NIH Stem Cell Task Force administrative office
- NIH Stem Cell Task Force administrative office compiles responses from ICs and summarizes them in one spreadsheet
- The NIH Stem Cell Task Force will analyze the portfolio review to assess the current science, identify gaps, and determine new research opportunities
- This assessment will be shared with the participants of the stem cell workshop to provide the status of the NIH's current research portfolio

Convene State-of-the-Science Workshop (Winter 2008)

- Establish a State-of-the-Science workshop planning committee from members of the NIH Stem Cell Implementation Committee and members of the NIH Stem Cell Task Force
- Set priorities for the workshop

- Create statement of work for external contractor
- Decide who is responsible for each portion of planning the workshop
- Set due dates for completion of action items
- Hire logistics support contractor for the workshop who will: Contact NIH Events Management Staff to confirm room reservations for the dates selected by the planning committee, make arrangements for room set up, audiovisual requirements, and other meeting arrangements, determine ADA considerations, provide travel arrangement services to all invited participants, arrange hotel accommodations, arrange payment/reimbursement of travel related expenses and finalize all logistics for the Symposium
- Identify speakers for the workshop
- Choose the workshop panel chairperson
- Finalize dates of the workshop
- Prepare Agenda
- Prepare brochures, posters, and advertisements
- Prepare meeting materials
- Finalize all logistics and hold Workshop
- Follow up with thank you letters to all of the participants
- Synthesize information from the workshop to determine what derivation technique may require additional basic or animal research
- Discuss next steps regarding initiatives
- Develop initiatives based on the information learned from the workshop

Hold Human Pluripotent Stem Cell Research (hPSC) Symposium to Prioritize Research with the Greatest Potential for Clinical Benefit (Spring 2008)

- Establish symposium planning subcommittee from members of the NIH Stem Cell Implementation Committee and members of the NIH Stem Cell Task Force
- Set priorities for the symposium
- Create statement of work for external contractor
- Decide who is responsible for each portion of planning the symposium
- Set due dates for completion of action items
- Hire logistics support contractor for the workshop who will: Contact NIH Events Management Staff to confirm room reservations for the dates selected by the planning committee, make arrangements for room set up, audiovisual requirements, and other meeting arrangements, determine ADA considerations, provide travel arrangement services to all invited speakers, arrange hotel accommodations, arrange payment/reimbursement of travel related expenses and finalize all logistics for the Symposium
- Identify speakers for the symposium

- Ask NIH scientific program officers from each IC supporting grants on pluripotent stem cells to suggest grantees for making oral presentations
- Choose the conference plenary session moderator
- Finalize dates
- Prepare Agenda
- Prepare brochures, posters, and advertisements
- Prepare meeting materials
- Finalize all logistics and hold Symposium
- Follow up with thank you letters to all of the participants
- Synthesize information from the Symposium to prioritize research with the greatest potential for clinical benefit
- Discuss next steps regarding initiatives
- Develop initiatives to encourage further research in pluripotent stem cell biology with the greatest potential for clinical benefit
- NIH will host additional symposiums to assess the progress and future promise of pluripotent stem cells

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