Alternative Sources of Human Pluripotent Stem Cells

A White Paper

The President's Council on Bioethics

ALTERNATIVE SOURCES OF HUMAN PLURIPOTENT STEM CELLS

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A White Paper of
The President's Council on Bioethics

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LETTER OF TRANSMITTAL TO

THE PRESIDENT OF THE UNITED STATES

The President's Council on Bioethics
1801 Pennsylvania Avenue, N.W., Suite 700
Washington, D.C. 20006
May 10, 2005

The President
The White House
Washington, D.C.

Dear Mr. President:

I am pleased to present to you *Alternative Sources of Human Pluripotent Stem Cells,* a White Paper of the President's Council on Bioethics.

Since the publication of our report, *Monitoring Stem Cell Research,* in January of 2004, the Council has continued to ponder and discuss the ethical challenges posed by human embryonic stem cell research and the demands of scientists to develop new human embryonic stem cell lines. While they may well in the future prove to be of considerable scientific and therapeutic value, new human embryonic stem cell lines cannot at present be obtained without destroying human embryos. As a consequence, the worthy goals of increasing scientific knowledge and developing therapies for grave human illnesses come into conflict with the strongly held belief of many Americans that human life, from its earliest stages, deserves our protection and respect.

Seeking to advance biomedical science while upholding ethical norms, the Council has taken a keen interest in recent suggestions that science itself might provide a way around this

ethical dilemma. Accordingly, we have been looking into ways of obtaining pluripotent, genetically stable, and long-lived human stem cells (the functional equivalent of human embryonic stem cells) that do *not* involve creating, destroying, or harming human embryos. We have found that there are, broadly speaking, four such possible approaches: stem cells might be obtainable from dead embryos; from living embryos, by non-destructive biopsy; from bioengineered embryo-like artifacts; and from reprogrammed adult somatic cells. In this White Paper, we introduce each of these four approaches and offer a preliminary analysis of their strengths and weaknesses, ethical, scientific, and practical.

While different members of the Council assess the merits of the four proposals differently, the Council shares the view that the group of proposals here discussed—and others like them that they may stimulate—deserve the nation's careful and serious consideration. We offer this White Paper both to enrich and inform public discussion of the ethical dimensions of stem cell research and especially to encourage scientists to explore these and other possible ways to press forward with pluripotent stem cell research in ways that all Americans can wholeheartedly support.

Mr. President, allow me to join my Council colleagues and our fine staff in thanking you for this opportunity to offer you and the American people our assistance in the critical efforts to promote a biomedical science that will simultaneously serve human needs and preserve human dignity.

Sincerely,

Leon R. Kass, M.D.
Chairman

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Preface

Alternative Sources of Human Pluripotent Stem Cells is a White Paper of the President's Council on Bioethics, which was created by President George W. Bush on November 28, 2001, by means of Executive Order 13237.

The Council's purpose is to advise the President on bioethical issues related to advances in biomedical science and technology. In connection with its advisory role, the mission of the Council includes the following functions:

- To undertake fundamental inquiry into the human and moral significance of developments in biomedical and behavioral science and technology.

- To explore specific ethical and policy questions related to these developments.

- To provide a forum for a national discussion of bioethical issues.

- To facilitate a greater understanding of bioethical issues.

The President left the Council free to establish its own priorities among the many issues encompassed within its charter and to determine its own modes of proceeding.

Stem cell research has been of interest to, and associated in the public mind with, this Council since its creation. Taking up the charge given to us by President Bush in his August 9, 2001,

speech on stem cell research, the Council has from its beginnings been monitoring developments in this fast-paced and exciting field of research. In January 2004, the Council published a report, *Monitoring Stem Cell Research,* which provided an overview of the law, ethics, and science of stem cell research. That report was intended to serve as a source of clear, intelligible, and useful information for both policymakers and the general public regarding the current state of this important research and of the debates that surround it.

Much of the ethical controversy over stem cells derives from the fact that, until now, the only way to obtain human pluripotent stem cell lines has been to derive them from living human embryos by a process that necessarily destroys the embryos. If a way could be found to derive such stem cell lines without creating and destroying human embryos, a good deal of that ethical controversy would subside.

The present White Paper may be regarded as a new contribution to the stem cell discussions. It reports on some recent developments that deserve public notice because of their potential for finding a morally uncontroversial means of obtaining pluripotent human stem cells. Over the past six months, the Council has been looking into specific scientific proposals for obtaining pluripotent, genetically stable, and long-lived human stem cells by methods that would not involve destroying or endangering human embryos. In December 2004, the Council heard presentations of two such proposals, one by Drs. Donald Landry and Howard Zucker of the Columbia University College of Physicians and Surgeons, and the other by Dr. William Hurlbut of Stanford University (and a member of this Council). In March 2005, the Council discussed a staff working paper in which these two proposals, as well as two others, were explained and analyzed.

That staff working paper, extensively revised and improved in light of Council discussions and member comments, is what the Council is now issuing as a White Paper, *Alternative Sources of Human Pluripotent Stem Cells.* In its present form, the White Paper has also benefited from expert review by three prominent scientists (Andrew Fire of the Stanford University School of Medicine, Markus Grompe of the Oregon Health & Science University, and Janet Rossant of the Samuel Lunenfeld Research Institute in Toronto, Ontario), as well as consultation with scientists at the National Institutes of Health.

The White Paper introduces the four proposals and begins an analysis of their strengths and weaknesses, ethical, scientific, and practical. Because the scientific and practical merits of these proposals are in large part empirical matters, not settled in advance by mere speculation, we give special weight to the ethical analysis. We also explore, in a preliminary way, whether these alternative avenues of deriving and using pluripotent stem cells are likely to be embraced by scientists or to become eligible for federal funding.

It remains to be seen whether any of these proposals will succeed scientifically, and more discussion is surely required on some of the ethical issues we have identified. Nevertheless, having conducted this "preliminary hearing," we believe that several of these possibilities have sufficient merit to commend them now to wider public attention and further scientific investigation. People of all moral and political persuasions should be pleased to learn that scientists and others are creatively seeking morally unproblematic and uncontroversial ways to advance this promising area of scientific research.

In creating this Council, President Bush expressed his desire to see us

> consider all of the medical and ethical ramifications of biomedical innovation. . . . This council will keep us apprised of new developments and give our nation a forum to continue to discuss and evaluate these important issues. As we go forward, I hope we will always be guided by both intellect and heart, by both our capabilities and our conscience.

It has been our goal in the present White Paper, as in all of our work, to live up to these high hopes and noble aspirations.

LEON R. KASS, M.D.
Chairman

Alternative Sources of Human Pluripotent Stem Cells

Introduction

Human embryonic stem cells hold great interest because of their pluripotency—their capacity to give rise to the various specialized cells of the body—and because of their longevity—their ability to be propagated for many generations in laboratory culture without losing their pluripotency. Until now, these cells have been obtainable only from living human embryos [at the 100-to-200-cell (blastocyst) stage of development] by a process that necessarily destroys the embryos and that therefore makes this research ethically controversial. Over the past several years, the ethical controversy has been the subject of federal (and state) legislation and public policy and of ongoing public debate.[*]

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[*] Since 1995, Congress has annually enacted legislation (the Dickey Amendment) that prohibits the use of federal funds for research in which human embryos are destroyed or harmed. In 2001, President George W. Bush instituted the current policy, which permits federal funding for research on those embryonic stem cell lines already in existence, but not for derivation or use of any new lines (the creation of which would require *new* embryo destruction). Vigorous debates continue about the ethics of embryonic stem cell research, as well as about the federal funding policy. For a synoptic view of these ethical and political discussions, including a history of the relevant public policy decisions, see *Monitoring Stem Cell Research: A Report of the President's Council on Bioethics* (2004), especially chapters two and three. For the text of the Dickey Amendment, see endnote 10.

The President's Council on Bioethics is committed to the goals of advancing biomedical science and upholding ethical norms. Notwithstanding our sometimes sharp individual ethical differences, we have recognized that all parties to the debates about embryo research have something vital to defend, and not only for themselves but for all of us.[1] As members of a national public bioethics body, we are also mindful of the need to understand and respect the strongly held ethical views of our fellow citizens, even when we do not share them. For these reasons, we must be receptive to any creative scientific or technical suggestions that might enable scientists to proceed with their research in ways that would not raise ethical questions or violate the ethical principles of many Americans.

Accordingly, in an effort to find ethically uncontroversial ways to advance human embryonic stem cell research, the Council has recently been looking into specific proposals for obtaining pluripotent, genetically stable, and long-lived human stem cells by methods that would meet the moral standard of not destroying or endangering human embryos in the process. This White Paper introduces these proposals and begins an analysis of their strengths and weaknesses, ethical, scientific, and practical. Because the scientific and practical merits of these proposals are in large part empirical matters, not settled in advance by mere speculation, we give special weight to the ethical analysis. We also explore, in a preliminary way, whether these alternative avenues of deriving and using pluripotent stem cells are likely to be embraced by scientists or to become eligible for federal funding.

Conceptually, four broad approaches present themselves. The stem cells could be derived: (1) by extracting cells from embryos already dead; or (2) by non-harmful biopsy of living embryos; or (3) by extracting cells from artificially created non-embryonic but embryo-like cellular systems (engineered to lack the essential elements of embryogenesis but still capable of some cell division and growth); or (4) by dedifferentiation of somatic cells back to pluripotency. In each of these four cases, the scientific standard by which success should be measured is only the desired *functional capacity* of the cells derived—stable pluripotency—and *not* their *origin* (embryos, adults, or artificial embryo-like clusters of cells). Should stem cells obtainable by one or another of these methods turn out to have exactly the same properties and capacities as embryonic stem cells (ESCs), their value for scientific research should be no different from that of standard ESCs.

Recently, more or less detailed examples of each of these four approaches have been proposed or discussed.

According to the *first* proposal, pluripotent human stem cells are to be derived from early IVF embryos (roughly 4-8 cells) that have spontaneously died (as evidenced by the irreversible cessation of cell division) but some of whose blastomeres* appear normal and healthy. Crucial to this

* A "blastomere" (literally, a part of a "blast" or embryo) is a cell contained within an early embryo (up to two days after conception), which comprises a small number of such blastomeres. Thus a 4-celled embryo contains four blastomeres, and an 8-celled embryo contains eight blastomeres. Beyond the 8-cell stage, on the third day after conception, the early embryo turns into a compact sphere known as a

approach is (a) enunciating a concept of organismic death of an early embryo and (b) devising criteria that permit a determination that embryonic death has occurred. In addition, to satisfy the moral standard, only those once-frozen embryos that are thawed and that die spontaneously *during efforts to produce a child* will be eligible for post-mortem cell extraction. This proposal was presented at the Council's December 3, 2004, meeting by Drs. Donald Landry and Howard Zucker of the Columbia University College of Physicians and Surgeons.

According to the *second* proposal, pluripotent stem cells are to be derived from blastomeres obtained by biopsy of an early human embryo. Crucial to this approach is finding a stage of early embryonic development at which (a) the removal of one or a few cells by biopsy can be carried out without harming the embryo, while (b) the cell or cells removed from the embryo are usable as a source of pluripotent stem cells.

The *third* approach comprises a variety of proposals for engineering "biological artifacts" possessing some of the developmental capacities of natural embryogenesis (but lacking the organismal character of human embryos) and containing cells from which pluripotent stem cell lines can

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morula; and on the fifth day after conception, the morula becomes a blastocyst (100-200 cells), a hollow ball of cells surrounding an inner cell mass. The cells of an embryo at the morula or blastocyst stage, being more differentiated than those of a 4-8-cell embryo, will not be referred to here as blastomeres, although some authors use the term to describe any cells of an embryo before it cavitates to become a blastocyst.

be derived. Crucial to this approach is demonstrating both (a) that the developing entity is truly not a human embryo and (b) that the cells derived from it are in fact normal human pluripotent cells. In addition, one must show that creating such biological artifacts does not itself introduce other ethical problems. One such proposal ("Altered Nuclear Transfer") was presented at the Council's December 3, 2004, meeting by Council Member Dr. William Hurlbut.

The *fourth* proposal 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 "dedifferentiate" them back into pluripotent stem cells. Crucial to this approach is discovering a way to reverse cell differentiation all the way to pluripotency, but not (as in cloning) even further back to totipotency.*

This White Paper describes each of these proposals and examines them with a view to the following three questions:

Ethical Question: Is it ethically sound? That is, would the proposed method overcome the ethical objections that have been raised against current methods of deriving embryonic

* A totipotent cell (for example, the fertilized egg or zygote) is one that can give rise to the entire organism, including the extra-embryonic membranes; a pluripotent cell (for example, an embryonic stem cell) is one that can give rise to many if not all the different cell types of the human body, but *not* to the whole organism as a living integrated entity.

stem cells that entail the destruction of human embryos? And does the proposed method raise new ethical difficulties of its own that make it problematic, worrisome, or even unacceptable?*

Feasibility Question: Is it scientifically sound? That is, might it reliably produce stable, pluripotent stem cell lines of sufficient quality for biomedical research and, in due course, for clinical trials in human beings?

Practical Question: From the perspective of both public policy and research practice, is this proposal "realistic"? That is, are there good reasons for believing that, if it is found to be scientifically feasible, it might be adoptable—by scientists as useful, by policy makers as legally eligible for federal

* The discussions in this paper take for granted the existing practices of assisted reproduction, including in vitro fertilization, the storage of frozen embryos for later reproductive use, and preimplantation genetic screening and diagnosis (PGD) of in vitro embryos prior to their transfer to initiate a pregnancy. These practices raise ethical issues of their own, and some people (including some Council members) object to them altogether. Although we recognize that there are many, and often deep, questions connected with the growing control over all aspects of human procreation, we will (for the most part) not be analyzing or arguing those questions here. We recommend, however, that they not be lost sight of, especially as the political acceptability of some of the proposals reviewed here will be influenced by where people stand on those larger questions. And clearly, some people will evaluate the proposals here under review not solely in themselves, but also by assessing their relationship to and potential effect on the way assisted reproduction is practiced, and by judging whether the proposed uses of dead embryos, blastomere biopsy, egg harvesting, and altered nuclear transfer create incentives for engaging in practices they deem misguided, unethical, or unwise.

support, and by the people and their elected officials as morally worthy of federal support? (In considering these questions, as we will in each case below, we are not to be understood either (a) to be making predictions about what scientists *will in fact* believe or (b) to be offering recommendations for what policy makers *should in fact* do. The first must await the arrival of relevant options; the second must depend on an assessment of the ethical claims and on prudential judgments to be made in specific circumstances that we cannot predict. Still, practical people will want to know whether any of this could become useful scientific practice or wise public policy, and we are obliged to discuss some aspects of these questions.)[*]

[*] Even if one or more or of these alternative sources of pluripotent stem cells were to meet all these requirements (ethical soundness, scientific feasibility, eligibility for federal funding, and broad acceptability to scientists), there would still remain the question of whether the cost of pursuing such alternatives—the necessary investment of scientific energy and resources, and the possible diversion of such energy and resources from other promising avenues of research—would outweigh the benefits. This White Paper does not pretend to answer that serious question, for it cannot be answered a priori, in advance of knowing empirically those costs and benefits. Moreover, how any person answers that question will depend on how strongly he or she cares about the additional "benefits" and "costs" of requiring scientific research to respect certain moral boundaries and strongly held moral qualms, in this case about protecting the moral worth of embryonic human life.

I. Pluripotent Stem Cells Derived from Organismically Dead Embryos (Landry-Zucker Proposal[2])

This proposal begins by making a close analogy with the use of human cadavers for biomedical research or as sources of organs. Today, we find morally and socially acceptable the removal (with consent) of vital organs from no-longer-living developed human beings once they have been declared dead.[3] Therefore, Landry and Zucker suggest, we should also find morally and socially acceptable the removal (with consent) of materials for stem cell derivation from no-longer-living *undeveloped* human beings (human embryos) once they have been declared dead. Applying the traditional concept of death—the irreversible loss of the integrated functioning of an organism as a whole—to the earliest stages of human life, Landry and Zucker propose a *concept of organismic death* for the early-stage human embryo: the irreversible loss of the capacity for "continued and integrated cellular division, growth and differentiation."* As the *criterion for determining organismic death* of an embryo produced by in vitro fertilization (IVF), they propose the "irreversible cessation of cell division in the embryo observed in vitro."

After fertilization in vitro, a high percentage of human embryos that reach the 4- or 8-cell stage undergo

* From reference 2: "We propose that the [minimal] defining capacity of a 4- or 8-cell human embryo is continued and integrated cellular division, growth and differentiation. We further propose that an embryo that has irreversibly lost this capacity, even as its individual cells are alive, is properly considered organismically dead."

spontaneous "cleavage arrest"—that is, their cells simply stop dividing. The vast majority of these arrested embryos do not resume cell division, never form blastocysts, and are incapable of successfully implanting in the uterus. In most cases, spontaneous cleavage arrest is associated with chromosomal abnormalities in the cells of the developing embryos. Yet some of the arrested embryos turn out to be "mosaic"—that is, some of their cells exhibit chromosomal abnormalities, while others appear to be (chromosomally, at least) normal blastomeres. It is these normal-appearing blastomeres in cleavage-arrested, mosaic embryos that may turn out to be a source of embryonic stem cells. Landry and Zucker propose that those embryos that have undergone *irreversible* cleavage arrest should be declared organismically dead and hence suitable (with proper consent) for harvesting of blastomeres for stem cell derivation.*

To identify when organismic death occurs in IVF embryos, Landry and Zucker propose a two-part research strategy. First, they propose a "natural history" study to determine just when a non-flourishing embryo in vitro ceases cell division irreversibly:

> Previously frozen early embryos that have failed to divide within 24 hours of thawing and are [therefore]

* The cells of an organismically dead embryo have stopped dividing altogether; their cleavage arrest is considered *irreversible* in the sense that the cells show no tendency to resume dividing *while remaining part of the dead embryo*. Of course, the goal of the Landry-Zucker proposal is to retrieve from these dead embryos some cells that can be induced to *resume* dividing *under conditions conducive to stem cell derivation*.

> no longer wanted [because they are no longer fit] for their original reproductive purpose are observed every few hours for several additional 24-hour periods.* After observing several hundred embryos, the time beyond which no arrested embryo resumes division can be determined. One can reasonably conclude that embryos that have not divided by this period will not divide at any later time, i.e., they are organismically dead.

Second, they propose an experimental study to attempt to identify physical or biochemical cellular markers that correlate with the arrest of cell division and in whose presence any arrested embryo could be declared dead:

> [IVF] Embryos declared [organismically] dead could then be characterized for secreted or cell surface markers or spectroscopic signatures that correlate with the arrest of cell division. These markers and signatures could then be tested for their predictive value. In this manner the criteria for determining the death of a human embryo could be refined.

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* It might be asked why Landry and Zucker confine their attention to embryos that have been frozen and then thawed, as opposed to "fresh" never-frozen embryos. The answer is that, in current IVF practice, healthy-looking embryos that have reached the 4- or 8-cell stage but are not selected for transfer to the uterus are generally frozen for possible future transfer attempts. To delay freezing such embryos while looking for signs of cleavage arrest would, arguably, subject the embryos to additional risk, contrary to the intention of the proposal. In contrast, the embryos of interest to Landry and Zucker—frozen embryos that are thawed out but never resume cell division—are not, in current IVF practice, either transferred to the uterus or frozen a second time.

According to Landry and Zucker, determining criteria of organismic death through the natural history study would be sufficient to commence efforts to derive human embryonic stem cells from dead embryos; the subsequent experimental study could facilitate this effort by rendering the determination of embryonic death more certain and reliable.

A. Is It Ethically Sound?

The Landry-Zucker proposal is based on an attractively simple ethical idea: it should be permissible to harvest cells from embryos that have died (provided of course that their deaths have not been caused or hastened for such purposes). Yet the final ethical assessment of this proposal will depend very much on exactly how it is implemented, within the practices and protocols of IVF. Several other pertinent questions have been raised as well.

1. *Can we be certain that the IVF embryos used in this proposal are really dead?*

This challenge unites two separate concerns, one about knowing whether the criteria for death have been met in any particular case, the other about the criteria themselves.

Landry and Zucker propose to harvest cells from 4- or 8-cell thawed human embryos that have irreversibly ceased to divide. Since the harvested cells are to serve as a source of stem cells, it is not feasible to wait for the death and dissolution of each and every cell before declaring the

embryo dead and eligible for use in research. To be useful for the project, the arrested embryos must contain at least some viable cells that retain normal developmental potential. Some of these cells, for example, might resume dividing if extracted and placed in the proper milieu. How, then, can we be sure that such an embryo is really dead? More generally, can we confidently declare that an embryo is dead just because all of its cells have stopped dividing? What exactly do we mean by the "organismic death" of an embryo?

It must be acknowledged that the concept of organismic death—the death of an organism *as a whole*—has not been commonly applied to an embryo, that is, to any largely undifferentiated organism so close to the beginning of its life. Moreover, even granting the applicability of the concept, identifying the *criteria for determining* organismic death for an embryo is, at least for now, more difficult than it is for an adult, owing to the absence of any integrating vital organs. Unlike a person at the end of life, an embryo has no identifiable controlling organ—no brain to be considered "brain dead," no heart that has ceased to beat and circulate the blood. For now, our judgment that an embryo has lost "integrated function as an organism" must be based simply on the observed absence of coordinated cell division. It is partly for that reason that Landry and Zucker hope that studies might reveal biochemical markers that would put the phenomenological finding of *irreversible* cleavage arrest on a sounder, "more objective" footing.

Yet despite these uncertainties, as Landry and Zucker suggest, the life of an embryo (as of any organism) *is* more

than just the sum of the lives of its constituent cells; and the death of an embryonic organism-as-a-whole is not the same thing as the death of its constituent cells. For the embryo to develop as an integrated organism, there must be an integrated set of internal signals directing the differentiation, development, and growth of the multicellular organism *as a whole*. The breakdown or absence of that set of signals amounts to the death of the developing embryo, manifested most clearly in the irreversible cessation of coordinated, organized cell division. Just as a person can be declared dead even while some of his organs and cells continue for a time to function and grow, so an embryo can undergo organismic death even while some of its individual cells may remain alive *as cells*, capable of further division if isolated and placed in a suitable environment.

Landry and Zucker point to studies showing that a substantial proportion of IVF embryos, after thawing, *never exhibit any further cell division,* even though some of their blastomeres appear otherwise normal. It is these embryos, they suggest, that can be unambiguously declared "organismically dead," and whose normal-looking blastomeres may be suitable candidates for embryonic stem cell derivation.[*]

[*] Yet here, too, a caution must be observed. As discussed elsewhere in this paper (see section 2 on p. 29, as well as endnote 18), we do not know precisely when, in embryonic development, human blastomeres cease to be totipotent, that is, individually capable of growing into a complete human being. To avoid the possibility that the cells extracted from a dead embryo might be totipotent, it would perhaps be advisable to carry out the Landry-Zucker proposal using somewhat older

2. *Will not this proposal put embryos at additional risk?*

The Landry-Zucker proposal involves scrutinizing thawed IVF embryos for signs of death and extracting cells from organismically dead embryos. A reasonable concern is that this procedure should not expose any living human embryos to risks they would not ordinarily encounter in the practice of assisted reproduction. Sharing this concern, Landry and Zucker place special strictures on which embryos are to be used, both in natural history studies to determine the criteria of death and in subsequent stem cell derivations. The only embryos that would be considered for use would be those (1) that were originally created with reproductive intent, (2) that were thought healthy enough to be kept alive in cryostorage for possible second or third child-producing attempts, and (3) that, after thawing, turned out, alas, to be dead. The natural history research proposed by Landry and Zucker would simply continue to watch those embryos that were not making any developmental progress, in order to determine more precisely exactly when they were irreversibly incapable of further development. No living embryo would be subject to manipulative intervention or to any procedure that increased its exposure to harm. No new or extra embryos would be created for such research, and no embryo would be deliberately killed or otherwise exposed to harm. This "death watch" study proposed by Landry and Zucker seeks only to discover which embryos are already dead, not to induce weak or doomed embryos to die.

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cleavage-arrested embryos (containing 8 cells or more) rather than embryos as early as the 4-cell stage.

3. *Changing practices and incentives for IVF practitioners*.

The proposed experiments to determine the natural history of organismic death in IVF embryos should be done in a way that does not compromise the safety of, or make more physically or psychologically onerous, current IVF clinical procedures. They also should not change incentives and practices regarding embryo production. Some people worry that approving the use of dead embryos for stem cell derivation will lead to the creation of even more embryos than are now produced in excess of reproductive need, precisely so that some could be allowed to die for the sake of getting stem cells from them. Yet it is important to note that, under the Landry-Zucker proposal, embryos that divide normally upon thawing, but are allowed to die by a human decision (not to transfer them into a woman's uterus), would *not* be eligible for donation. Precisely in order to avoid participation or complicity in the death of any embryos, their proposal restricts use to only those embryos that fail altogether to divide upon thawing and that have thus died "on their own." For now, no change of practice or incentives would be likely or necessary, since many embryos are thawed for a second reproductive trial and, of these, a sizeable fraction (in some cases, close to one half) fail to develop.[4] Still, going forward it will be important to provide oversight and assurance that the desire for material useful for the natural history study or for stem cell derivation does not increase either the number of frozen embryos that are thawed in an attempt to produce a second pregnancy or the number created for reproductive purposes in the first place. It would be an ethically dubious innovation if the implementation of the

Landry-Zucker proposal were to change the incentives or the practices of IVF embryo creation or storage in these ways.*

4. *Issues for informed consent*.

Additional discussions with the embryo-producing, child-seeking patients that explain (and seek consent for) the proposed experiment and use will be required. And a fitting informed-consent form will have to be developed and approved. While this is well within the scope of present practice, it will necessarily involve discussion of the likely death of some embryos created by the IVF procedure. Many clinicians shy away from using the word "death" to describe what happens to the embryos that do not develop in vitro, fearful that such a designation would imply that those embryos were in fact alive and that they might therefore be held at fault for their resulting deaths. Nevertheless, only a frank discussion of these facts could produce meaningful consent from the patients. Discussion and suitably documented agreements will also be needed to address additional questions, including who owns the commercial rights to any human pluripotent stem cell line created by this research.

* To turn this morally scrupulous proposal into morally scrupulous practice would seem to require protocols and enforceable regulations that would provide very strict monitoring and oversight. The political acceptability of the Landry-Zucker proposal might hinge on having a regulatory system in place so that there would be some way to track IVF embryos (as the Canadians do), thus ensuring that abuses do not creep in.

5. *For ethical purposes, is there a sufficiently strong analogy between harvesting cells from dead embryos and harvesting organs or removing tissues from dead persons?*

Landry and Zucker base the ethical justification of their proposal on the analogy with end-of-life organ donation. Yet the analogy is not altogether exact: unlike the physician caring for a dying patient, the IVF clinician does not in general treat the death of an embryo as a grave matter. Certain standard IVF procedures, including superovulation and cryopreservation of excess embryos, knowingly increase the likelihood that many individual embryos will die or be discarded;[5] but this is not generally considered a reason to forgo production and freezing of "extra" embryos. Some observers, troubled by these aspects of the clinical context in which this proposal would be carried out and disinclined to benefit by complicity in these practices, do not find the analogy with organ donation sufficiently compelling to justify the extraction of cells from dead IVF embryos, especially when the intended use of the extracted cells is scientific research rather than the immediate use of organs to save dying patients.

Nonetheless, an exact analogy with organ donation is not required to show that what Landry and Zucker are proposing is what they claim it to be: a *morally preferable* alternative to the intentional destruction of embryos. Death comes spontaneously to many embryos, both in vivo and in vitro, and it is difficult to see how dissecting spontaneously dead embryos can be said to harm them. And if the principle "Once dead, then usable"—of course, with informed consent and showing respect for the corpse—

works ethically for removing transplantable organs or research materials from dead adult human beings, it should work equally well for dead human embryos (provided, again, that the embryos are indeed dead and that their prospective users have not deliberately killed them or neglected them so that they would die). In the end, whether this proposal proves to be *morally acceptable* and practically wise remains to be seen.

B. Is It Scientifically Sound?

The Landry-Zucker proposal has yet to be tested, though it is technically possible to begin testing it immediately, not only in animals but also in humans. Three basic questions need to be answered: Can objective markers of organismic death be found? Can pluripotent stem cells be derived from dead embryos? If so, will they be chromosomally (and otherwise) normal?

1. *Can one find objective markers for organismic death?*

It seems likely that the natural history study will identify "duration without cleavage" as one objective marker of embryonic organismic death, but the exact criterion has yet to be established.[*] Whether additional biochemical markers can be identified is not yet known, but, according to Landry and Zucker, their discovery is not

[*] "Failure to cleave 24 hours after thawing" may not prove to be a sufficient criterion for organismic death, since, in the study by Laverge, et al. [see endnote 4], roughly 10% of embryos meeting that criterion showed some sign of further cleavage by 48 hours after thawing.

essential for the immediate implementation of the proposal. Ultimately, the issue of markers is largely an empirical question: one will not know the answer until the effort to find them is made.

2. *Can one in fact get usable pluripotent stem cells from dead embryos?*

Once embryos in vitro have been determined to be organismically dead, a basic scientific question, still unanswered, is whether pluripotent stem cells can then be derived, starting from any remaining blastomeres. There is reason to believe that some cells in arrested embryos may retain their developmental potential, which can be reactivated by transferring them to the appropriate milieu; but the evidence is very preliminary.[6] In addition, recent work by Dr. Nicolai Strelchenko and colleagues (working at Chicago's Reproductive Genetics Institute headed by Dr. Yury Verlinsky) has described the production of human pluripotent stem cells derived by culturing blastomeres removed from morula-stage (8-24-cell) human embryos.[*7] The next step would be to show that stem cells can be derived from single blastomeres extracted from 8-cell embryos.[†] It would then have to be shown that similar

[*] Note that most embryologists reserve the term "morula" for embryos of more than 8 cells. See footnote starting on page three above.

[†] Deriving stem cells from isolated single blastomeres may prove significantly more challenging than deriving them from disaggregated blastocysts or morulae; in human ESC derivations achieved so far, groups of cells have been cultured together, and it is not known whether the presence of other cells is necessary for the derivation of embryonic stem cells from a single blastomere.

results can be obtained using blastomeres extracted from organismically dead IVF embryos.

3. *Will the stem cells derived from dead embryos be normal and healthy?*

Questions have been raised regarding whether pluripotent stem cell lines isolated from organismically dead IVF embryos would be abnormal, and in particular, aneuploid (that is, having more or fewer than the normal number of chromosomes).[8] The answer cannot be given in advance, but there are reliable methods for determining it in every case. Each isolated pluripotent cell line would be grown in vitro, so that a detailed study could subsequently be done on the chromosome complement of each cell line. Such testing would identify those pluripotent stem cell lines with a normal chromosome complement; repeated karyotype testing throughout the period of laboratory culture and storage could confirm that the chromosome complement remains normal. Moreover, while pluripotent stem cell lines with a normal chromosome complement would have the broadest potential therapeutic applicability, pluripotent stem cell lines with specific abnormal chromosome complements (for example, three copies of chromosome 21, as is observed in people with Down syndrome) could be useful in basic studies of how (in this example) the presence of the extra chromosome affects differentiation processes that subsequently lead to a human genetic disease.

C. Is It "Realistic"?

Two kinds of practical questions have been raised: First, will scientists want to work with these cells? And, second, will the research be supportable by federal funding, under the legislative and administrative restrictions now in place?

1. *Scientific acceptance*.

Scientists understandably want to work only with the best materials. Why, it is asked, would they settle for cells derived from dead embryos, especially since embryos that die early are generally abnormal, either chromosomally or in other ways?* And why should they bother trying to develop these cells lines, when they can use existing ESC lines or derive new ones at will from living IVF ("spare" or newly created) embryos? One answer is that they would welcome such cell lines if research using them were eligible for federal funding. But a better answer, and on the main question, is this: there is simply no way to know in advance whether cells derivable from dead embryos are in fact in any way inferior to cells derivable from still living blastocysts. One must do the experiment and see.

* Council Member Janet Rowley has suggested that it would be strange, while allowing large numbers of unwanted but otherwise normal and viable IVF embryos to die, to ask scientists to make strenuous efforts to rescue cells, potentially normal but potentially abnormal, only from those thawed embryos that have *spontaneously* stopped dividing.

2. *Eligibility for federal funding*.

The Landry-Zucker proposal aims to provide a basis for future attempted isolations of pluripotent human stem cells by a procedure in which no embryos are killed for the purposes of research. Only embryos that are found to have died in the context of standard IVF clinical procedures would be used in attempts to produce the stem cells. Such experiments would ordinarily be considered human tissue research and require local IRB approval. After that, most states would permit the research.[9] It is somewhat more difficult to determine whether experiments to evaluate and implement the Landry-Zucker proposal would be eligible for federal funding under current law and policy. Two federal policies are particularly relevant: the Dickey Amendment[10] and President Bush's embryonic stem cell policy statement of August 9, 2001.[11] The purpose of the Dickey Amendment is to deny federal funds for any experiment in which living human embryos are killed or harmed, while the intent of the President's policy is to promote embryonic stem cell research without sanctioning or encouraging *future* destruction of human embryos[*]; both policies will have to be re-examined in the context of organismically dead IVF embryos. Assuming Landry-Zucker criteria for embryonic death are established and

[*] The President's August 9, 2001, policy (see endnote 11), which offers federal funding for research on embryonic stem cell lines only if those lines were derived before the date of the policy, is intended "to allow us to explore the promise and potential of stem cell research without crossing a fundamental moral line, by providing taxpayer funding that would sanction or encourage further destruction of human embryos that have at least the potential for life."

clinically met, it would appear that federal funding for the further manipulation of such dead embryos would not violate either the letter *or the spirit* of the Dickey Amendment.* It is less clear whether the same could be said of the natural history studies to determine the precise criteria for embryonic organismic death, especially because federal funding has never been available for IVF research or practice or any treatment whatsoever of ex vivo embryos.† Nevertheless, a strong argument can be made that the mere observation of embryos that had spontaneously ceased to divide hardly constitutes doing them harm or causing their death. Whether the President's policy and budget for federal funding of stem cell research would—or should—be expanded to support either research to derive stem cells from dead embryos or research on stem cell lines already derived from dead embryos with private support is a question whose answer will depend not only on the issue of legal eligibility but also on the assessment of other ethical, scientific, and prudential considerations of the sort we have just discussed.

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* The use of federal funds for the derivation of stem cells from doomed but still-living embryos (the so-called "spare" embryos, unwanted for transfer in efforts to produce a child) violates the letter of the Dickey Amendment; the use of federal funds for research on embryonic stem cells derived by *someone else's* prior destruction of a living embryo violates the spirit of the Dickey Amendment. Deriving stem cells only from already dead embryos seems not to commit either of these violations.

† The Dickey Amendment has been enacted with the support of many members of Congress who are unwilling to "take for granted" the practices mentioned in the footnote on page six, including freezing of embryos or even IVF itself.

II. Pluripotent Stem Cells via Blastomere Extraction from Living Embryos

Pluripotent stem cell lines could, in theory, be derived starting from small numbers of cells ("blastomeres") removed from *living* human embryos. Is there a stage of early human embryonic development at which cells, capable of developing in vitro into pluripotent stem cells, can be extracted without harming the embryo's prospects for developing into a live-born child?*

Blastomere extraction from living IVF embryos is currently performed to conduct what is called "preimplantation genetic diagnosis" (PGD). PGD is a procedure increasingly being used in conjunction with assisted reproductive technologies to test IVF embryos for genetic and chromosomal abnormalities prior to uterine transfer for beginning a pregnancy. PGD generally involves removal of a blastomere or two from living 6-8-cell embryos, and subsequent genetic tests on the removed blastomeres. Following the genetic screening, the desired embryos, from which one or two blastomeres have been removed, are then transferred to women to initiate pregnancy. Although estimates vary widely, one recent report suggested that more than 1,000 babies had been born

* At present, embryonic stem cells are typically derived by extracting cells from the inner cell mass of the embryo at the blastocyst (roughly 100-cell) stage; this entails the destruction of the trophectoderm (that is, the outer ring of cells in the spherical blastocyst structure, the precursor of the fetal contribution to the placenta) and the death of the embryo.

worldwide following PGD.[12] Thus, apparently normal children have been born following removal of one or two blastomeres from the 6-8-cell embryo. However, long-term studies to determine whether this procedure produces subtle or later-developing injury in children born following PGD have been recommended[13] and are sorely needed.

As indicated above, Dr. Nicolai Strelchenko and his colleagues have shown that embryonic stem cells can be derived from human embryos containing 8-24 cells (see reference 7). In their method, *all* the cells of the embryo are disaggregated and cultured on feeder cells (and the embryo is killed in the process). It may be some time before stem cell lines can be reliably derived from *single* cells extracted from early embryos, and in ways that do no harm to the embryo thus biopsied. But the initial success of the Verlinsky group's efforts at least raises the future possibility that pluripotent stem cells could be derived from single blastomeres removed from early human embryos without apparently harming them.*

A. Is It Ethically Sound?

1. *Harm to the embryo?*

With the Landry-Zucker proposal, the major ethical issue concerned the question of whether the embryos would in fact be truly dead. Here, the major ethical issue

* A similar idea was proposed by Representative Roscoe Bartlett of Maryland as far back as 2001.

concerns the question of possible harm (and perhaps also benefit) to the still-living embryo whose cells are removed. Removal of blastomeres from developing IVF embryos in vitro is currently done primarily in the context of avoiding pregnancies at risk for genetic disease. Toward that end, the PGD techniques are used to test a group of embryos with a view to identifying those embryos that can be transferred to the woman without carrying known markers for genetic disease. (The embryos that do carry the abnormal genes are discarded.) Strictly speaking, embryo biopsy as currently practiced in PGD cannot be said to be undertaken for any future child's benefit, since the procedure does not directly help those embryos that are ultimately implanted. Also, the genetically healthy embryos that are transferred to initiate a pregnancy will have been subjected to the as-yet-unknown risks of the blastomere biopsy procedure. For many individuals and couples, the known short-term and potential long-term risks of the PGD technique are thought to be more than balanced by the desire of the couple to have their own biological child free from a specific genetic disease. Others believe this practice is unethical, since it involves discriminating against genetically disabled embryos and ultimately discarding them. There also remains substantial debate about the ethical propriety of using PGD in two specific cases: (1) to identify embryos that would give rise to children who could serve as compatible bone marrow donors for sick siblings,[14] and (2) to determine the sex of the embryos in order to be sure that only embryos of the desired gender were transferred.[15]

How would the ethical analysis change if living embryo blastomere extraction were to be performed not for PGD but for stem-cell derivation? Assuming that single blastomeres extracted from early embryos could in fact be used to derive pluripotent human stem cells, would this procedure pass ethical scrutiny? Since the removal of a cell or two from the embryo is not (usually) fatal, the individual embryo that is biopsied is not killed. However, since the blastomere extraction is not being performed for the good of the embryo, it might be hard to justify the procedure ethically. As Dr. Gerald Schatten told the Council in December 2002, "Embryo biopsy is a complicated technique, and it's a very expensive technique, and it's not clear that it is completely innocuous. So you would not go into embryo biopsy unless there were compelling reasons for actually going through all of the costs and expense and heroics of ART [assisted reproductive technologies]."[16] And even when prospective parents do elect to use ART, subjecting otherwise healthy embryos to biopsy procedures in order to derive stem cells seems ethically troubling. Indeed, even if the biopsied embryo and the resulting child were not physically harmed, a strong line of moral argument might still lead one to object on the grounds that the embryo is being treated merely as a means to another's ends. For there are more ways to do injustice to another human being than by actions that do discernable or manifest harm. Using human beings for purposes of no benefit to them and without their knowing consent is one such injustice, even if doing so results in no evident or eventual harm to body or psyche.

Such worries or objections might be moderated should the blastomere removal be undertaken, at least in part, for the possible benefit of the future child. As noted earlier, in embryo biopsy for PGD, sometimes two blastomeres are removed. This raises the futuristic possibility that one cell could be used for genetic diagnosis, while the other cell is used to derive a line of stem cells genetically autologous to the embryo and the child it becomes. Looking still farther ahead, patients using IVF *without* concern for genetic disease (and hence not interested in ordinary PGD) might nonetheless consider blastomere removal solely for the purpose of deriving immunologically compatible stem cells for their future child. Some might consider such a practice ethically justified on the grounds that the child born after uterine transfer of the embryo might later derive medical benefit from the existence of a genetically matched line of pluripotent stem cells, stored in case he needs it for future disease therapy. Others, however, doubt the wisdom of exposing the prospective child (while an embryo) to a hazardous procedure merely for the sake of some hypothetical future benefit, or of encouraging parents to practice embryo biopsy simply or mainly as a source of "personalized" stem cells should their future child someday have need of them.[17] Besides, genetically matched stem cells can be more effectively derived using the newborn's umbilical cord blood (a well-established procedure), though it is unclear whether the stem cells isolatable from cord blood will have all the same capacities as embryonic stem cells.

2. *Are the removed blastomeres not themselves the equivalent of embryos or capable of developing into them?*

Another possible source of ethical concern has to do with the totipotency of early-stage human blastomeres in vivo.[18] After the first cleavage of the fertilized human egg in vivo, both resulting blastomeres are capable of forming a complete embryo that grows into a child.[*] It is not certain at what point in embryonic development in vitro (as in IVF) such totipotency of the blastomeres disappears; it may be that, by the 8-cell stage, sufficient differentiation has taken place that individual human blastomeres are no longer individually totipotent without aggregating them or combining them with other early embryos. Clearly, however, if the blastomere removed for biopsy has the potential to develop into an embryo and a child on its own, some would find destruction of that blastomere ethically objectionable. And, in any case, little would have been gained ethically if the goal of the entire enterprise was a non-controversial procedure for deriving stem cells that did so while avoiding destruction of living embryos.

[*] Identical twinning can apparently take place during at least two stages of the in vivo development process: Spontaneous separation of the blastomeres at the two-cell stage leads to the formation of twins with two separate placentas (about 1/3 of cases); while embryo splitting at the blastocyst stage leads to the formation of identical twins that share the same placenta (about 2/3 of cases).

3. *May one perform non-harmful blastomere extraction on embryos that are NOT going to become children?*

If embryo biopsy proves to be a usable source of pluripotent stem cells, some might argue that it would be ethically permissible to carry out such a procedure—not fatal and perhaps not harmful at all—not only on embryos that were soon to be transferred to a woman, but also on IVF "spare" embryos that are *not* ultimately selected for uterine transfer. Indeed, because of the still unknown risk of harm from blastomere removal to the child subsequently emerging from a biopsied embryo, some have suggested that the biopsy procedure can be ethically done *only* on an embryo that is definitely not going to become a child. Others, however, consider any proposed utilitarian treatment of such embryos to be morally unacceptable, since it necessarily classifies "spare" (and still living) embryos as ethically available for research uses.

4. *Can the research necessary to test this proposal be conducted in an ethically acceptable manner?*

Learning how to implement this proposal—even for the variation that would only seek stem cells that might eventually benefit the child whose embryonic beginning was biopsied to obtain them—has its own ethical difficulties. Techniques would have to be developed for deriving pluripotent stem cells, not only from whole early-stage human embryos, but also from individual blastomeres extracted from an embryo. It may prove difficult to develop and refine those techniques without

exposing many human embryos to death and injury.* If embryo biopsy is to be embraced as a *morally uncontroversial* way to derive stem cells, the research needed to test the proposal and perfect the technique would have to avoid killing or harming human embryos. It is far from clear that the necessary research can be accomplished with this restriction in place. Even if a perfected technique could someday derive stem cells from single blastomeres harmlessly obtained, the failure to satisfy this *testing-stage* ethical requirement could render this option ethically little better than the currently controversial methods for deriving embryonic stem cells.

5. *Changing the practices of assisted reproduction*.

There is ethically more at stake in this proposal than the fate of individual biopsied embryos. There are also large issues raised by the direct intrusion of research objectives into the practice of reproductive medicine, *while* it is being practiced (that is, in the moments when decisions about embryo transfer are still being made). The Landry-Zucker proposal would make available for stem cell derivation only those embryos that had already died. In contrast, this embryo-biopsy proposal would manipulate still-living embryos immediately destined for reproductive transfer. From the perspective of the would-be parents, is it really better for them (and for their child-to-be) if those performing PGD are concerned not only with good diagnosis but also with procuring useful cells for a research

* Indeed, in the initial studies showing the potential usefulness of morula-stage human embryos as a source of stem cells, many human embryos were obviously destroyed.

colleague? Decisions about how many and which embryos to transfer are often made in stressful circumstances, where timing is critical and the doctor's attention is limited. Bringing scientists (or considerations of research) into this process, for reasons having nothing to do with the well-being of parents and their children-to-be, would seem to be a dubious intrusion. It would also make assisted reproduction seem even more like manufacture, a process with many side uses and side benefits, rather than simply a way to help people have children. To say the least, much careful planning and oversight would be needed to prevent the research interest from adversely affecting the way reproductive medicine is practiced and the meaning it has for the larger society.

B. Is It Scientifically Sound?

The recent work of Strelchenko and colleagues with disaggregated 8-24-cell embryos suggests that whole human embryos as early as the 8-cell stage are potentially usable as a source of pluripotent stem cells. This work would have to be reproduced and refined before we could be certain that embryos at such an early stage are indeed a dependable source of stem cells. It would then have to be shown that stem cells can also be derived from isolated blastomeres extracted from an 8-cell embryo.[*] It seems far

[*] Studies with mouse embryos have shown that isolated 8-cell blastomeres will develop into vesicles of trophectoderm, containing little or no inner cell mass, making derivation of ESC lines difficult. And there is no reason to believe that things will be different in humans. However, there has been one published report claiming

from certain that enough cells can be extracted from the embryo to derive stem cells while also avoiding injury to the embryo.

On the question of whether biopsy can be safely performed on early stage embryos, there is mixed evidence from animal studies. Krzyminska and colleagues, working with mouse embryos, found that "biopsy had the least impact when performed at the 8-cell stage." When performed on 8-cell embryos, they found that biopsy did not significantly impair development in vitro or the rate of implantation after transfer; but compared to intact embryos, fewer biopsied embryos (52% versus 71%) resulted in viable fetuses.[19] Because human PGD is such a novel, still small, and as-yet-unstudied practice, we do not have good data for the implantation rate for biopsied—as compared with non-biopsied—human embryos.

In any event, since apparently healthy children have been born after embryo biopsy at the 8-cell stage,[*] it would

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derivation of mouse ESC lines from isolated 8-cell blastomeres: One cell line was obtained from 52 dissociated 8-cell stage embryos. (See Delhaise, F., et al., "Establishment of an embryonic stem cell line from 8-cell stage mouse embryos," *European Journal of Morphology* 34, 237-243 [1996].) The Council is grateful to Dr. Janet Rossant for these observations.

[*] Granting that apparently healthy children have been born following IVF and PGD, does removal of one or two blastomeres from the early human embryo have *no effect* on the child who is later born? We cannot be certain. There is some evidence (at least in mice) for asymmetrical division and distinct cell fates at the early cleavage stages. (See Piotrowska-Nitsche, K., et al., "Four-cell stage mouse blastomeres have different developmental properties," *Development* 132, 479-490 [2005].)

appear to be possible to safely remove one or two blastomeres from an 8-cell embryo in order to try to generate a line of pluripotent human stem cells. Only further research and effort can settle the matter.

C. Is It "Realistic"?

Let us assume that a stage of human embryonic development can be identified at which cells can be removed without injuring the still-living embryo, that a non-injurious procedure for removing the cells is perfected, and that the cells can then be used to derive pluripotent stem cells. Will scientists want to work with these cells? And will the stem cell research be supportable by federal funding, that is, both eligible under the legislative restrictions and embraceable by administrative policies now in place?

1. *Scientific acceptance.*

Whether this approach is likely to be adopted by scientists in the future as a way to produce pluripotent human stem cell lines depends on several unknowns, central among them the efficiency of the process (for example, how many good stem cell lines are obtainable from how many biopsied embryos, and at what cost of effort and expense). A further crucial consideration will be

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It is possible that the loss of one or two blastomeres is entirely rectified, but even if development proceeds in a healthy manner, it may be that the child born is somehow a different child than the one that would have resulted from an undisturbed embryo.

the properties of the pluripotent stem cells that are produced by blastomere disaggregation of early embryos. If the resulting pluripotent stem cells turned out to be as good as or better than the current human embryonic stem cell lines (derived from the inner cell mass of blastocysts, a later stage of embryonic development), prospects for using this approach would improve. If, however, the proposal were deemed ineligible for federal funding, the prospects for this approach would not look very good, given that there are several other well-established methods for producing human embryonic stem cells.

2. *Eligibility for federal funding*.

An argument could be advanced that this approach complies with the Dickey Amendment, as long as the embryos biopsied are truly not harmed. But there is today insufficient evidence to determine whether biopsied embryos are, by virtue of the procedure or the removal of cells, in fact at risk of harm. Thus, although any derived pluripotent cell lines might be eligible for funding, doing the prior research *to derive them* might not be. A second obstacle facing such prior research would be the longstanding Congressional opposition to all federal funding of any activities involving in vitro fertilization. Whether the President's policy and budget for federal funding of stem cell research would—or should—be expanded to support research on stem cell lines derived (with private support) from biopsied embryos is a question whose answer will depend not only on the issue of legal eligibility but also on the assessment of other ethical,

scientific, and prudential considerations of the sort we have just discussed.

III. Pluripotent Stem Cells Derived from Biological Artifacts*

Under this heading are various proposals to construct a biological artifact, lacking the moral status of a human embryo, from which pluripotent stem cells could then be derived. For example, Council Member William Hurlbut has advocated what he calls "altered nuclear transfer" (ANT), a procedure that, if successful, would offer a way to produce pluripotent stem cells within "a limited cellular

* Here, as in so many other ethically charged situations, terminology matters enormously. One must take special pains not to prejudice consideration of the ethical issues by choice of terms that, intentionally or unintentionally, incline readers and hearers one way rather than another. This case is no exception. Precisely because the purpose here is to create an entity that is *not* in fact a human embryo, but that is nevertheless enough *like* a human embryo that it too contains cells that can be cultured to give rise to pluripotent stem cells, the artificially produced entity must be at once "non-embryonic" yet "embryo-like." But if the product is referred to as an "embryo-like" or (by analogy to "android") "embryoid" body, people may be encouraged to overemphasize its resemblance to an embryo and to slight the claim that this product is decidedly not a human embryo. On the other hand, if the product is referred to as a "non-embryonic" body or structure, people may be encouraged to think that its desired non-embryonic character has in fact been achieved. Since a major ethical issue turns on whether or not the biological artifact is truly not a living human embryo, all prejudgment by terminological fiat should be, where possible, avoided. We therefore usually call it (merely) a "biological artifact," denoting its origin but leaving its essential status open.

system that is biologically and morally akin to a complex tissue culture."[20] This proposal, as yet untested experimentally (even in animals), is conceptually based on modifying the procedure of somatic cell nuclear transfer (SCNT), now used to produce cloned embryos. In standard SCNT, a somatic cell nucleus is introduced into an oocyte (egg cell) whose own nucleus has been removed. The product is a cloned embryo (virtually identical, at least genetically, to the organism from which the donor nucleus was taken), the functional equivalent of a fertilized egg that is capable (at least in some cases) of developing into all later stages of the organism. ANT, the modified procedure proposed by Hurlbut, involves altering the somatic cell nucleus *before* its transfer to the oocyte, and in such a way that the resulting biological entity, while being a source of pluripotent stem cells, *would lack the essential attributes and capacities of a human embryo*. For example, the altered nucleus might be engineered to lack a gene or genes that are crucial for the cell-to-cell signaling and integrated organization essential for (normal) embryogenesis.[21] It would therefore lack organized development from the very earliest stages of cell differentiation. Such an entity would be a "biological artifact," not an organism. Removal of cells from, or even disaggregation of, this artifact would not be killing or harming, for there is no living being here to be killed or harmed. After extraction from this artifact, the cells could have the missing gene or genes reinserted, with a view to deriving "normal" pluripotent stem cells from them.

A. Is It Ethically Sound?

In offering his proposal for ANT, Hurlbut emphasizes that no embryo would ever be created or destroyed; since the genetic alteration is carried out in the somatic cell nucleus before transfer, the biological artifact is "*brought into existence* with a genetic structure insufficient to generate a human embryo." Hurlbut compares the product of ANT to certain ovarian teratomas and hydatidiform moles, genetically or epigenetically abnormal natural products of failed fertilization that are not living beings but "chaotic, disorganized, and nonfunctional masses." If, as Hurlbut suggests, the biological artifact is ethically equivalent to a tissue culture, teratoma, or mole, there would seem to be nothing ethically problematic about harvesting stem cells from it. Nonetheless, a number of ethical questions and concerns have been raised about this proposal.

1. *Would not this "artifact" really be a very defective embryo*?

Some people have wondered about the accuracy of the claim that no embryo creation or destruction is entailed by the proposal. They understand that the proposed biological artifact has, from the beginning, a built-in genetic defect that prevents it from developing normally. Yet they worry that this is not the production of a non-human entity but the deliberate creation of a doomed or disabled human embryo, or, in other words, that Hurlbut's proposal amounts to creating and using "bad or sick embryos," rather than "non-embryonic entities." Hurlbut's claim that his method would *not* yield a defective embryo rests on the

fact that a genetic alteration sufficient to prevent embryogenesis is introduced into the nucleus *before* it is transferred to the oocyte, and that the alteration would be so fundamental that it would preclude the integrated organization that characterizes a human embryonic organism. If no embryo is created, then none is violated, mutilated or destroyed (which would be the case if the alteration were introduced after normal fertilization).

Nonetheless, some critics wonder how the product of that nuclear transfer is in fact essentially different from—and less an embryo than—a fertilized egg into which the same disabling genetic alteration is introduced only *after* normal fertilization. A person's perception of the truth in this matter may depend on how easy it is to turn the genetic defect on or off. The easier it is to activate and deactivate the genetic defect, the more this proposal *looks like* interfering with the normal development of an embryo rather than manufacture of an artificial non-organismic structure. Unless it can be shown that the artifact is *not* truly an embryo—that is, that it lacks (by design) the possibility of becoming not only a live-born human but an organized, differentiating early human embryo and fetus—there will likely be ethical debate on whether it is permissible to continue "abusing" the embryo-like entity by suppressing the genes it needs for development. Furthermore, even if the artifact were *conclusively* shown to lack genes indispensable for becoming an *organized*, *differentiating* human embryo, some critics might continue to insist that it was destined to become a *defective*, *severely deformed* human embryo, the defect and deformity having been deliberately inflicted on it by the scientist.

Experimental work in animals, however, might help resolve these questions and allay these concerns. If, for example, the biological artifact begins to grow in ways that resemble unorganized cells in a tissue culture, critics may gain confidence in the non-embryonic character of the product.

2. *The ethics of egg procurement*.

Like ordinary cloning-for-biomedical-research (SCNT), this altered nuclear transfer proposal requires a (probably large) supply of human oocytes, which would have to be donated, purchased, or produced for research purposes. Some will find this troubling, and on multiple grounds. Obtaining human oocytes currently requires hormonal stimulation and superovulation in the women who would be donating or selling their eggs, practices that carry significant medical risks to the women, risks not easily justified when they themselves or their prospective children are not the beneficiaries of the oocyte retrieval (as are women undergoing these procedures in hopes of having children). In addition to the medical risks, there are also ethical concerns about the practice of commercializing human reproductive tissue and about any buying and selling of eggs: the exploiting of poor women, the coarsening of society's sensibilities, the developing of markets in (reproductive) human tissues. More deeply, one critic suggests, we must consider the implications and the consequences of coming to regard human eggs and sperm as fungible raw materials, to be used in ways that have nothing to do with their procreative biological and human meaning. There is a risk that, in seeking to avoid the

problem of embryo destruction, we would thus be furthering a dehumanized and utilitarian view of human beginnings as bad as the one that this alternative proposal was trying to combat.[22]

There is, at least in theory, the possibility that human oocytes can be obtained not from women egg donors by superovulation but from ovaries surgically removed from patients or harvested from cadavers. The ooocyte precursors extracted from these ovaries could then be matured in vitro. Alternatively, the ovaries could be transplanted into animal hosts and eggs produced by hormonal stimulation of the animals.[23] Research in this area is at a very preliminary stage. And the objections just noted to non-reproductive uses of human reproductive tissue could also be raised to obtaining eggs in these non-invasive ways, should they ever become possible.

3. *Ethical concerns about ANT itself.*

To some observers, the procedures involved in ANT are inherently objectionable. Certain commentators, for example, find the very idea of tampering to put something destructive into the human genome, even for a good cause, morally and aesthetically offensive. Some find it aesthetically repulsive and ethically suspect to be *creating* such neither-living-nor-nonliving, near-human artifacts, a practice they regard as ethically no improvement over *destroying* early embryos. Other critics of the ANT proposal argue that, while it is ethically acceptable to modify the human genome for treatment (with consent) of individuals with known genetic disorders, it is quite another thing to

do so for other than therapeutic purposes or to do so in eggs or sperm before there is an existing needy individual. Some think this is a major ethical boundary that ought not to be crossed lightly.[24] Others are troubled by the attitude of mastery or hubris implied in a project that aims at engineering a human biological artifact.

In response to these objections, Hurlbut replies that the ANT technique would be used only for serious scientific research within the frame of therapeutic purposes beneficial to a large population whose medical needs are of grave concern. Further, he points out that we accept many medical and research practices that are aesthetically ugly and morally worrisome, from cutting into a living body or brain, to giving people a dose of disease for vaccination, to growing great sheets of skin from cells harvested from foreskins. We do these things—and many others like them—in the service of the higher goal of healing, the very goal to which the ANT proposal is dedicated.[25]

4. *Concerns about ANT on "slippery slope" grounds.*

Several worries have been expressed not about the proposal itself but about what it might lead to, or about what it might be seen as justifying in the future. For some, the proposal appears to open the door to a troubling new field of biomedical engineering. True, its initial relatively modest goal is only to produce a biological artifact capable of yielding pluripotent human stem cells while not itself being a complete human organism with developmental potential. And if, in the process, definite criteria could be established for distinguishing between the not-human and

the human, this proposal might have the salutary effect of erecting boundaries that would open avenues for scientific advance without threatening human dignity, boundaries that do not now govern the practices of human embryo research in the private sector. But, extrapolating into the future from an ANT precedent, pursuing this proposal could—whether intentionally or not—help to launch a new field of bioengineering, devoted to manufacturing intermediate biological forms that are sufficiently human to yield useful biomedical materials, but not so human that it would be unethical to destroy or exploit them. Hurlbut's arguments could be adapted to justify the deliberate production of teratomas, hydatidiform moles, inter-species hybrids, and other ill-formed, non-viable, but potentially useful biological artifacts. Once we start down the road of deliberately engineering artificial entities with some human properties, it is not obvious how bright ethical boundaries between the acceptable and the unacceptable can be drawn.

A second "slippery slope" concern has to do with the flexibility of the developmental stage at which disordered growth is set to begin. Hurlbut's proposal involves building in a genetic alteration that causes development to go irretrievably awry from the very start of embryonic development. But suppose a useful genetic modification were achieved that entailed chaotic and disorganized development only at a *later* stage of embryonic (or even fetal) development. Could not the *ethical reasoning* in defense of ANT be used to argue that such further-developed but still inherently defective entities are "fetus-like but not actual fetuses," and hence ethically suitable for exploitation and destruction? (The same question is

relevant for ongoing destructive embryo research using normal IVF embryos, whose exploitation and destruction are justified because of the human benefits they might eventually bring.) It would certainly be troubling if the ethical case for ANT could be used to justify the creation and destruction of fetus-like entities. Hurlbut's proposal, seeking a source of pluripotent human stem cells, confines its attention to the early stages of embryonic development. But someone looking for a source of tissues or even primordial organs might be tempted to apply his reasoning to later and later stages of development, not excluding the deliberate production of anencephalic fetuses or even newborns, useful as a source of organs and tissues. Hurlbut's criterion for being a truly human organism—"organization of the species-typical kind"—would appear to be inherently malleable and open to interpretation (and even mischief).

Arguments that worry about future extensions of present techniques, or future applications to dubious ends, or future uses of current ethical justifications to validate later unsavory practices, while worth considering, tend to assume that people are either unable or unwilling to draw the necessary distinctions and erect the necessary ethical boundaries between current acceptable practices and future unacceptable ones—an assumption that is readily subject to dispute. But since the truth of this assumption *cannot* be known in advance, and can only be demonstrated case by case and then only by going forward and running the predicted risks, there is at least some reason to wonder whether any newly devised technological solution to the ethical problem of embryo destruction will not, in the end,

be creating or contributing to ethical problems worse than the one it set out to cure.

B. Is It Scientifically Sound?

Although the proposal for ANT has yet to be tested, several scientists have indicated that they believe that it can easily be made to work, and a few are apparently ready to try it out in non-human animals. It would be crucial to show that the disorganizing genetic or epigenetic alteration introduced into the somatic cell nucleus before transfer could be fully controlled, with predictable results, and fully reversed without residual abnormalities in the derived cells extracted from the embryo-like artifact. For unless the genetic engineering were fully reversible, the resulting stem cells would likely carry genetic or other alterations that might compromise the value of the stem cells. Moreover, there may be a tension in this proposal between ethical considerations, which require that insurmountable developmental barriers be genetically built into the embryo-like entity from the start, and technical feasibility, which would favor simpler barriers to development that are easily and completely reversible. Presumably, the more hard-wired the introduced defect is, the more difficult it will be to reverse. However, it is quite possible that, with a sufficiently determined research program, even the more technically daunting versions of the proposal could be achieved.

It is important to note that both some of the strengths and some of the weaknesses of the ANT proposal come

from the fact that it is basically a form of SCNT or cloning. On the positive side, if successful, ANT could provide stem cells with a much greater diversity of genotypes than is possible under current methods of stem cell derivation (or under the other two proposals we have considered so far*), allowing a far wider range of medical possibilities such as disease modeling or drug testing. Likewise, ANT would allow the generation of pluripotent stem cell lines with pre-engineered alterations (such as enhanced immune response or correction of a genetic defect) that might make them of more scientific interest or therapeutic value.

On the negative side, however, attempts to clone mammals have so far resulted in high rates of death, deformity, and disability in the animals that come to birth following SCNT. In 2002, research in the laboratory of Rudolph Jaenisch at MIT showed that, in cloned mice, about 4% of genes function abnormally, owing mainly not to mutations but to departures from normal activation or expression of certain genes.[26] It is not yet known whether similar genetic or epigenetic abnormalities will also be found in any stem cell lines that might be derived by ANT. Such problems are much less likely to be encountered in stem cell lines derived from IVF embryos.

* This advantage would, however, be shared by the next proposal, somatic cell dedifferentiation.

C. Is It "Realistic"?

1. Scientific acceptance.

Compared to deriving human embryonic stem cells from normal blastocysts, procedures such as ANT are quite complex and would yield cells that would then have to be restored to genetic normality before stem cell lines could be derived. Many scientists, we suspect, would be reluctant to attempt such challenging feats with no rational purpose other than to satisfy the ethical objections of others, and one prominent scientist in the stem cell and cloning field has recently made such a complaint publicly (notwithstanding the fact that the company for which he works has filed a patent application for precisely such a procedure).[27] Other scientists have reacted to news of the ANT proposal by describing it as exceedingly complex and technically challenging, not even testable without time-consuming experiments involving substantial investment of precious resources.[28] Even if federal funding for research on ANT-derived stem cell lines were approved, stem cell scientists might prefer to seek private funding of unrestricted stem cell research rather than follow procedures that seem to them burdensome and scientifically useless. Also, as Hurlbut himself acknowledges, proof of principle and safety-and-efficacy experiments need first to be done in animals, and it might be many months or even years before this process could be perfected using human tissues. Many stem-cell scientists, eager to press forward, are unlikely to wait for these new lines, especially if they are not themselves bothered by the embryo destruction that necessarily results when stem cell

preparations are derived, as they are now, from unused IVF embryos. On the other hand, there may be some scientists, either opposed themselves to destroying embryos or hoping to find a way around the current federal funding restrictions, who would be willing and even eager to test Hurlbut's proposal in animals, and several have apparently volunteered their collaborative services for such animal trials.

2. *Eligibility for federal funding.*

If the biological artifacts created and destroyed under this proposal were persuasively shown not to be human embryos, the proposal would presumably be deemed consistent with the Dickey Amendment and therefore eligible for federal funding. Whether the President's policy and budget for federal funding of stem cell research would—or should—be expanded to support research on ANT or other biological artifacts (even in animals) is a question whose answer will depend not only on the issue of legal eligibility but also on the assessment of other ethical, scientific, and prudential considerations of the sort we have just discussed.

D. Pluripotent Stem Cells via "Parthenogenesis."

Besides Hurlbut's ANT proposal, other methods of constructing embryo-like artificial structures are under investigation, including a technique recently demonstrated by Karl Swann and colleagues at the University of Wales College of Medicine, in which a human oocyte is

biochemically "tricked into thinking it has been fertilized."[29] The treated eggs divide to the blastocyst stage (50-100 cells), at which point stem cells can presumably be derived.[30] Although it undergoes several cycles of cell division, the "parthenogenetic" (that is, unfertilized but still developing) blastocyst-like entity is assumed by most commentators to lack entirely the potential for development as a human being, and is therefore, arguably, not really an embryo.* If this is correct, then this technique might provide another means for deriving pluripotent stem cells without creating or destroying embryos. Yet the only experiment that could prove whether this plausible assumption is in fact correct—transferring a parthenogenetic embryo to a woman to try to bring it to birth—cannot be ethically attempted. In the absence of such proof, the biological and moral status of the parthenogenetic blastocysts is likely to remain in doubt and controversial. Still, those who are convinced that parthenogenetic embryos have no chance of development beyond the blastocyst stage are likely to have few ethical objections to the production and use of such entities.[31] It remains to be seen whether viable and genetically stable pluripotent stem cells can be derived from these parthenogenetic blastocysts and whether imprinting and other issues related to their parthenogenetic origin might limit their utility in research or potential clinical trials. Under the present terms of the Dickey Amendment, this proposal would be unlikely to be eligible for federal

* However, live-birth parthenogenesis takes place naturally in certain amphibians and has been induced artificially even in mice. See Kono, T., et al., "Birth of parthenogenetic mice that can develop to adulthood" (Letters to *Nature*), *Nature* 428, 860-864 (2004).

funding, since the amendment specifically prohibits the use of federal funds for research that may cause harm to an embryo produced by, among other means, parthenogenesis.

IV. Pluripotent Stem Cells via Somatic Cell Dedifferentiation

A quite different route to the production of pluripotent stem cells would be to reprogram differentiated somatic cells so as to restore to them the pluripotency typical of embryonic stem cells. The obstacles here are not ethical, but technical. Because it involves neither the creation nor the destruction of human embryos, the common ethical objection to human embryonic stem cell research would not apply. But it would take new scientific advances and new technological innovation before such "dedifferentiation" of somatic cells back into pluripotent stem cells could be achieved. Several suggestions have, however, been offered for how such dedifferentiation might be achieved, and the value of success cannot be overstated. For if it were possible to undo the differentiation of somatic cells, running development in reverse back to the state of pluripotency, it would in principle be possible for autologous pluripotent stem cells to be obtained from the body of *any* human being. Such individualized stem cells would then be available as a potential source of personalized, immuno-compatible regenerative therapies.

A. Is It Ethically Sound?

There would seem to be nothing to object to ethically if procedures were developed to turn somatic cells into pluripotent stem cells, non-embryonic functional equivalents of embryonic stem cells. Of course, if the dedifferentiation were pursued beyond (mere) pluripotency to the point of yielding a totipotent cell—in effect, a cloned human zygote—the moral status of such a cell would become a serious issue, as would the permissibility of using it either for reproductive or for research purposes. For a totipotent cell is, arguably, an organism at the unicellular stage, and a strong case could be made that the product is not a pluripotent stem cell but an *embryo*.

B. Is It Scientifically Sound?

Research into dedifferentiation of somatic cells is at a preliminary stage, and it is much too early to know whether this will succeed. It may prove possible to culture specific populations of somatic cells—cells that may be especially susceptible to dedifferentiation—under conditions that might get them to reverse their differentiating epigenetic changes, thereby leading them to become more multipotent or even completely pluripotent; there is also some hope of identifying and isolating the chemical factors present in oocytes and other cells (such as ESCs) that are responsible for maintaining or restoring cells to pluripotency, and of using these chemicals to

dedifferentiate ordinary somatic cells (without the further need for oocytes or embryos).

In nature, limited dedifferentiation is involved in the regeneration of missing limbs in amphibians, though the precise mechanism is not yet known.[32] Studies have shown that some adult human somatic cell types (blood, liver, muscle) can be chemically dedifferentiated back into their corresponding multipotent progenitor cells (that is, adult stem cells).[33] Several research laboratories have reported the direct isolation of cells from bone marrow of children or adults that, when cultured in vivo, have or acquire the capacity to differentiate into many mature cell types, including cells originating from all three embryonic germ layers.[34] These cultured human multipotent cells also show the presence of certain biochemical properties ordinarily found only in human embryonic stem cells. It is interesting to speculate that it may be the same bone marrow stem cells, cultured in vitro under different conditions, that revert in some cases to (rather modestly multipotent) mesenchymal stem cells, in some other cases (further back) to the clearly multipotent adult progenitor cells, and in still other cases (yet to be achieved) to the *ur*-primordial stem cell, the fully pluripotent stem cell, functionally equivalent to an embryonic stem cell (though not of embryonic origin). If such "graded dedifferentiations" are indeed the cause of the variations seen among the cultivated stem cells now known to arise from bone-marrow stem cells, further research—using also stem cells obtained from umbilical cord blood—might very well turn out to yield the big payoff: fully pluripotent stem cells, obtainable at will and

altogether without any involvement of embryos—and well suited for autologous transplantation.

Another possible approach to somatic cell dedifferentiation relies on knowledge that might be gained through cloning-for-biomedical-research. In SCNT or cloning, a somatic cell nucleus is reprogrammed back to totipotency by transfer into an enucleated oocyte. Presumably, cytoplasmic factors that are present in the oocyte (and that may also be present in cultured embryonic stem cells*) are responsible for the dedifferentiation that takes place. If and when these cytoplasmic factors can be identified and isolated, it may be possible to use them—instead of SCNT into oocytes—to coax some ordinary somatic cells to dedifferentiate back to the pluripotent stage.[35] Once again, should the process of dedifferentiation go too far, back to totipotency, the end result will not be a stem cell but the functional equivalent of a zygote, and one would be back in the ethical soup from which this proposal was intended to provide an escape. Great care would therefore have to be exercised to ensure that dedifferentiation, if and when it occurs, goes only so far and no further. Given the complexity of the process, and how little we now know about the factors that regulate differentiation and its opposite, it is not likely that this (second) approach will yield results in the near future.

* The Council is grateful to Dr. Markus Grompe for this observation.

C. Is It "Realistic"?

1. Scientific acceptance.

Certainly, dedifferentiation of somatic cells back to their corresponding progenitor cells will likely be welcomed as a powerful new way to produce large quantities of multipotent adult stem cells. If dedifferentiation is perfected to the point of yielding cells as pluripotent as embryonic stem cells, there is no reason to doubt that this procedure would be widely embraced and the cells obtained widely used. An additional—clinical—potential benefit of such cells would be that specialized cells derived from them (for example, heart muscle cells, nerve cells) could be reintroduced therapeutically into the patient from whom they were derived *without risk of immunological rejection*.

2. *Eligibility for federal funding.*

Because this research does not involve human embryos at any stage, it would not offend either the letter or the spirit of the Dickey Amendment. Aside from the concern that dedifferentiation might proceed too far (resulting in the functional equivalent of a zygote), there would appear to be no obstacle to, or reason to oppose, federal funding of research on dedifferentiation of somatic cells.

Conclusion

The United States has been engaged in a vigorous ethical debate about embryonic stem cell research, prompted by tensions between the desire for biomedical progress and respect for nascent human life. The scientific and medical promise of stem cells has generated enormous excitement among researchers and patient groups. The ethical issues raised by embryo research have roused considerable public attention and concern. Many people share the hope that stem cell research will eventually save lives and yield the promised remedies for numerous chronic illnesses. Many people (including many who are eager for regenerative medicine to succeed) share the concern that embryonic stem cell research depends on destroying human embryos and cheapens human life by creating and using it for experimentation.

Some people hope that stem cells derived from non-embryonic sources (known as adult stem cells) will turn out to be as good as stem cells derived from embryos, but it is too early to tell which sort of stem cells will be most useful for the treatment of which diseases. Some believe that current federal law and policy governing the funding of embryonic stem cell research are too restrictive, and that the existing embryonic stem cell lines will not prove adequate for the work ahead. Others believe that medical

progress must not be purchased by destroying human life, even at its earliest stages, and that creation of human embryos specifically for use in research should be outlawed.

Mindful of the moral weight of the arguments on the various sides of this controversy, and charged with finding ways for science to proceed while respecting moral norms, the Council has taken seriously a number of recent proposals and suggestions for techniques to derive new pluripotent stem cell lines in ways that might be ethically uncontroversial.

This White Paper has summarized several current proposals for obtaining pluripotent human stem cells that do not require destroying human embryos. In each case, we have examined whether the proposal is ethically sound, scientifically feasible, and practically "realistic." *The inquiry we have undertaken constitutes no more than a preliminary hearing*, designed mainly to see whether there are any *insuperable* ethical, scientific, or practical objections to further consideration of these proposals. Because all of these proposals are relatively new, the ethical issues they raise need more discussion, and much research would be needed before it became clear which of them, if any, would succeed. Likewise, further legal interpretation and sober political deliberation would be required to determine which of the proposals are, under current law, *eligible* for federal funding and which are *both* ethically and scientifically *deserving* of such official national support. We hope that our analyses of the ethical, scientific, and practical aspects of these proposals will contribute to a

more informed and comprehensive scrutiny of their respective merits.

The analyses of this White Paper, while preliminary, do lead to the following provisional assessments. The last proposal, dedifferentiating somatic cells back to pluripotency, seems ethically the most unobjectionable, but for now scientifically and technically uncertain; recent derivations (from adults) of relatively undifferentiated multipotent stem cell lines* may be an encouraging, albeit preliminary, step toward this goal. The first proposal, seeking to derive stem cells from organismically dead embryos, has yet to be tested, even in animals. But the natural history studies proposed could be undertaken forthwith and in an ethical manner, not only in animals but also in humans, and we might learn soon whether reliable objective criteria for determining death of IVF embryos can be developed. The second proposal, seeking to develop stem cells from blastomeres extractable from living embryos, is also now technically feasible, though large ethical difficulties remain, concerning especially the propriety of imposing risks of embryo biopsy and blastomere removal on the born child the embryo might become, solely for research of no benefit to him or her. The third proposal, seeking to derive stem cells from genetically engineered artificial entities, is technically the most demanding and ethically the most complex and puzzling. Even its proponents agree that it would need to be carefully tested in animals before any thought of human trials could be countenanced.

* See endnote 34.

Among these several proposals, the Council has no unanimous recommendation to make. Different Council members are drawn more to one or less to another of the four proposals. Each of us weighs the ethical issues differently. And we have differing views on which approach is likely to succeed technically or to be useful practically. A few of us may suspect that the quest for alternative sources of stem cells is misguided, and that we should continue using the embryos we have (or can produce directly) in order to get any new stem cell lines we need.

Yet on the limited *ethical threshold* question—"Does this proposal appear to meet a minimum ethical standard to justify further serious consideration and scientific exploration?"—the Council offers the following *provisional* conclusions.[36]

The first proposal, *deriving cells from organismically dead embryos*. Although it raises some serious ethical questions, we find this proposal to be ethically acceptable for basic investigation in humans, provided that stringent guidelines like those proposed by Drs. Landry and Zucker are strictly observed. The results of such investigations would help to determine whether the method would in fact prove ethically acceptable in the long run.

The second proposal, *blastomere extraction from living embryos*. We find this proposal to be ethically *un*acceptable in humans, owing to the reasons given in the ethical analysis: we should not impose risks on living embryos destined to become children for the sake of getting stem

cells for research. This approach could, of course, be attempted in animals, but we do not yet see how results from animal experimentation could alter this assessment of ethical propriety in humans. We do not expect this method to become ethically acceptable for human trials in the future.

The third proposal, *cells derived from specially engineered biological artifacts*. Because this proposal raises many serious ethical concerns, we do not believe that it is *at this time* ethically acceptable for trials with human material. Although a few of us are not eager to endorse even animal and other laboratory work investigating potential human applications, most of us believe the proposal offers enough promise to justify animal experimentation, both to offer proof of feasibility and utility and to get evidence bearing on some of the ethical issues. We find no insuperable ethical objections to pursuing this proposal in animal models, which is, we note again, all that the proponents now seek to do. The possibility of any future endorsement of trying this approach in humans will depend upon a more thorough ethical analysis made possible in part by animal experiments.

The fourth proposal, *cells obtained by somatic cell dedifferentiation*. We find this proposal to be ethically unproblematic and acceptable for use in humans, if and when it becomes scientifically practical, provided the line between pluripotency and totipotency can be maintained, as discussed in the ethical analysis.

Despite any differences among us about the merits of each proposal, the Council shares the view that the group of proposals here discussed—and others like them that they might stimulate—deserve the nation's careful and serious consideration. Because the Council is wholeheartedly committed *both* to the advancement of science for the betterment of humankind *and* to the defense of human freedom, dignity, and the value of human life, we are pleased *to endorse these proposals as worthy of further public discussion*, and we are pleased *to encourage their scientific exploration in accordance with the preliminary ethical judgments just offered*. A good part of our uncertainty today about the merits of these proposals rests on the paucity of available scientific evidence and of demonstrated technical prowess. Both enthusiasts and skeptics regarding these proposals should agree at least on this: that further empirical studies will be needed before the true potential of these proposals can be properly assessed.

We conclude by stressing that while these four proposals are the ones that seem most worthy of analysis and discussion at this time, it is altogether possible, indeed likely, that other avenues to human pluripotent stem cells not requiring the destruction of human embryos may be proposed or discovered in the future. By limiting ourselves to these current proposals, we do not intend to exclude any additional ones. On the contrary, we publish this White Paper to encourage scientists to creatively devise other and better proposals and to highlight the appeal of the larger purpose: to find ways to advance pluripotent stem cell research that all our fellow citizens can wholeheartedly support. That end is, in the view of the Council, a desirable

goal for our society and one that justifies making the extra effort to seek out, assess, and attempt new, ethically uncontroversial methods of stem cell derivation.

Endnotes and References

[1] President's Council on Bioethics, *Human Cloning and Human Dignity: An Ethical Inquiry*, July 2002, pp. xxx and 121. For discussion of these competing goods, see Chapter Six, "Ethics of Cloning-for-Biomedical-Research." See also Chapter Three ("Recent Developments in the Ethical and Policy Debates") of *Monitoring Stem Cell Research: A Report of the President's Council on Bioethics*, January 2004.

[2] Landry, D. W. and H. A. Zucker, "Embryonic death and the creation of human embryonic stem cells," *The Journal of Clinical Investigation* 114, 1184-1186 (2004). The transcript of the presentation and discussion of this proposal at the December 3, 2004 Council meeting is available online at www.bioethics.gov (see session 6). This proposal was anticipated in Grinnell, F., "Defining embryo death would permit important research," *The Chronicle of Higher Education* 49(36), B13 (May 16, 2003), and Grinnell, F., "Human embryo research: from moral uncertainty to death," *American Journal of Bioethics* 4, 12-13 (2004).

[3] Although there is a broad consensus on the ethics of posthumous organ donation, there are some who have raised questions about the adequacy of the criterion (typically, "brain death") by which death ("irreversible loss of integrated functioning of the person") is established. See *President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research. Defining Death: A Report on the Medical, Legal, and Ethical Issues in the Determination of Death* (Washington: The Commission, 1981); Veatch, R. M., "The Conscience Clause," in *The Definition of Death: Contemporary Controversies*, ed. S. J. Youngner, et al. (Baltimore, Md.: Johns Hopkins University Press, 1999), 137-160; Shewmon, D. A., "Brainstem death, brain death and death: a critical re-evaluation of the purported evidence," *Issues in Law and Medicine* 14, 125-145 (1998); and Shewmon, D. A., "Chronic brain

death: meta-analysis and conceptual consequences," *Neurology* 51, 1538-1545 (1998).

[4] Landry and Zucker (*op. cit.*) refer to a study by Laverge, et al., in which, "out of 166 frozen embryos thawed for further growth, 78 embryos remained arrested at 24 hours after thawing, and 71 showed no sign of further cleavage at 48 hours." See Laverge, H., et al., "Fluorescent in-situ hybridization on human embryos showing cleavage arrest after freezing and thawing," *Human Reproduction* 13, 425-429 (1998).

[5] D. H. Edgar and coworkers estimate that "the implantation potential of a population of embryos was reduced by ~30% by being subjected to cryopreservation." See Edgar, D. H., et al., "A quantitative analysis of the impact of cryopreservation on the implantation potential of human early cleavage stage embryos," *Human Reproduction* 15, 175-179 (2000).

See also the following studies cited by Laverge, et al. (in the paper mentioned in endnote 4): Camus, M., et al., "Human embryo viability after freezing with dimethylsulfoxide as a cryoprotectant," *Fertility and Sterility* 51, 460–465 (1989); Levran, D., et al., "Pregnancy potential of human oocytes—the effect of cryopreservation," *New England Journal of Medicine* 323, 1153–1156 (1990); Van Steirteghem, A., et al., "Cryopreservation of human embryos," *Bailliere's Clinical Obstetrics and Gynaecology* 6, 313–325 (1992); and Van der Elst, J., et al., "Prospective randomized study on the cryopreservation of human embryos with dimethylsulfoxide or 1,2-propanediol protocols," *Fertility and Sterility* 63, 92–100 (1995).

[6] Recently, animal studies have shown that some individual blastomeres, removed from embryos that have ceased developing, can survive, grow, and function normally if they are transplanted into a still-living embryo that is itself developing normally. For example, in their presentation to the Council, Landry and Zucker pointed out work by Byrne and coworkers that showed that cells from abnormal partial blastulae of cloned amphibian embryos could be "rescued" by grafting them to normal host embryos where they contributed to several tissues. See Byrne, J. A., Simonsson, S., Gurdon, J. B., "From intestine to muscle:

nuclear reprogramming through defective cloned embryos," *Proceedings of the National Academy of Sciences USA* 99(9), 6059-6063 (April 30, 2002).

[7] Strelchenko, N., et al., "Morula-derived human embryonic stem cells," *Reproductive BioMedicine Online* 9(6), 623-629 (2004). See also U.S. Patent Application 20040229350, "Morula derived embryonic stem cells," filed November 18, 2004.

[8] The question was raised by Council Member Dr. Paul McHugh, at the December 2004 Council meeting. For discussion of this point, see the transcript of the December 3, 2004 meeting (session 6), available online at www.bioethics.gov.

[9] Studies reporting the derivation of human embryonic stem cell lines starting from excess blastocysts from IVF procedures and supported by *private funding* include: (1) Mitalipova, M., et al., "Human embryonic stem cell lines derived from discarded embryos," *Stem Cells* 21, 521-526 (2003), and (2) Cowan, C. A., et al., "Derivation of embryonic stem cell lines from human blastocysts," *New England Journal of Medicine* 350, 1353-1356 (2004).

[10] The Dickey Amendment, named for its author, former Representative Jay Dickey of Arkansas, has been attached to the Health and Human Services authorization bill each year since 1995. The provision reads as follows:

> ***SEC. 510.***
>
> *(a) None of the funds made available in this Act may be used for—*
>
> *(1) the creation of a human embryo or embryos for research purposes;*
>
> *or*
>
> *(2) research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death greater*

than that allowed for research on fetuses in utero under 45 CFR 46.208(a)(2) and section 498(b) of the Public Health Service Act (42 U.S.C. 289g(b)).

(b) For purposes of this section, the term 'human embryo or embryos' includes any organism, not protected as a human subject under 45 CFR 46 as of the date of the enactment of this Act, that is derived by fertilization, parthenogenesis, cloning, or any other means from one or more human gametes or human diploid cells.

The section of the Code of Federal Regulations (CFR) to which the Dickey Amendment refers reads as follows:

45 CFR §46.208 Activities directed toward fetuses* in utero *as subjects.

(a) No fetus in utero may be involved as a subject in any activity covered by this subpart unless:

(2) the risk to the fetus imposed by the research is minimal and the purpose of the activity is the development of important biomedical knowledge which cannot be obtained by other means.

The section of the Public Health Service Act to which the Dickey Amendment refers reads as follows:

§289g. Fetal research

(b) Risk standard for fetuses intended to be aborted and fetuses intended to be carried to term to be same

In administering the regulations for the protection of human research subjects which—

(1) apply to research conducted or supported by the Secretary;
(2) involve living human fetuses in utero; and
(3) are published in section 46.208 of part 46 of title 45 of the

Code of Federal Regulations or any successor to such regulations—

the Secretary shall require that the risk standard (published in section 46.102(g) of such part 46 or any successor to such regulations) be the same for fetuses which are intended to be aborted and fetuses which are intended to be carried to term.

The minimal risk standard governing research on fetuses is defined in the CFR as follows:

45 CFR §46.102

(i) Minimal risk *means that the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests.*

[11] See "Remarks by President George W. Bush on Stem Cell Research," as made available by the White House Press Office, August 9, 2001.

[12] Genetics and Public Policy Center, "*Preimplantation Genetic Diagnosis: A Discussion of Challenges, Concerns and Preliminary Policy Options Related to the Genetic Testing of Human Embryos,"* Washington, D.C. (2004).

[13] PGD can only be performed on embryos created through IVF. There are already questions about whether specific forms of IVF (in the absence of PGD) lead to an increased incidence of some rare genetic diseases. (See for example, Gosden, R., et al., "Rare congenital disorders, imprinted genes, and assisted reproductive technology," *Lancet* 361, 1975-1977 [2003].) In its 2004 report, *Reproduction and Responsibility*, the Council issued a recommendation to "undertake a federally funded longitudinal study of the impact of ARTs (Assisted Reproduction Technologies) on the health and development of children born with their aid."

[14] See, among other articles, Adams, K. E., "Ethical considerations of applications of preimplantation genetic diagnosis in the United States," *Biomedicine and Law* 22, 489-504 (2003); Rechitsky, S., et al., "Preimplantation genetic diagnosis with HLA matching," *Reproductive BioMedicine Online* 9, 210-221 (2004); Sheldon, S. and S. Wilkinson, "Should selecting savior siblings be banned?" *Journal of Medical Ethics* 30, 533-537 (2004).

[15] American Society for Reproductive Medicine, Ethics Committee Report, "Sex selection and preimplantation genetic diagnosis," *Fertility and Sterility* 72, 595-598 (1999); The Ethics Committee of the American Society of Reproductive Medicine, "Sex selection and preimplantation genetic diagnosis," *Fertility and Sterility* 82 (Suppl. 1), S245-248 (2004).

[16] See, transcript of the December 13, 2002 Council meeting (session 6), available online at www.bioethics.gov.

[17] Cohen, E., personal communication to the Council, commenting on this proposal.

[18] Can single blastomeres extracted from a 4- or 8-cell embryo ever give rise to a whole organism? For human blastomeres, the answer is not known, and there are substantial ethical objections to performing the experiments that could assess this question. In mice, apparently normal and fertile animals have been produced from single blastomeres isolated from a 4-cell embryo (and from two blastomeres isolated from an 8-cell embryo); but the blastomeres had to be cultured in the presence of cells from "carrier embryos." See Tarkowski, A. K., Ozdzenski, W., Czolowska, R., "Mouse singletons and twins developed from isolated diploid blastomeres supported with tetraploid blastomeres," *International Journal of Developmental Biology* 45(3), 591-596 (2001). In experiments with non-human primates (rhesus monkeys), Chan, et al., [see "Clonal propagation of primate offspring by embryo splitting," *Science* 287, 317-319 (2000)] have reported that two blastomeres isolated from an 8-cell embryo gave rise to a live-born monkey they called Tetra. See also Schramm, R. D. and A. M. Paprocki, "Strategies for the production of genetically identical monkeys by

embryo splitting," *Reproductive Biology and Endocrinology*, 2, 38 (2004).

[19] Krzyminska, U. B., Lutjen, J., O'Neill, C., "Assessment of the viability and pregnancy potential of mouse embryos biopsied at different preimplantation stages of development," *Human Reproduction* 5(2), 203-208 (1990).

[20] Quotations from Hurlbut are from his paper presented to the Council on December 3, 2004, "Altered Nuclear Transfer as a morally acceptable means for the procurement of human embryonic stem cells," available online in the December 2004 Council meeting background materials at www.bioethics.gov. For Council discussion of his paper, see transcript of the December 3, 2004 meeting (session 6), also available online at www.bioethics.gov.

[21] Chawengsaksophak, K., et al., "Cdx2 is essential for axial elongation in mouse development," *Proceedings of the National Academy of Sciences USA* 101(20), 7641-7645 (May 18, 2004).

[22] Cohen, E., *op. cit*.

[23] See, Gook, D. A., et al., "Oocyte maturation, follicle rupture and luteinization in human cryopreserved ovarian tissue following xenografting," *Human Reproduction* 18(9) 1772-1781 (2003).

[24] See, for example, the public comment made to the Council by Jaydee Hanson, representing the International Center for Technology Assessment, at the December 2004 meeting (session 7), the transcript of which is available online at www.bioethics.gov.

[25] For discussion of these issues, see transcript of the Council's March 4, 2005, meeting (session 5), available online at www.bioethics.gov.

[26] Humpherys, D., et al., "Gene expression in cloned mice derived from embryonic stem cell and cumulus cell nuclei," *Proceedings of the National Academy of Sciences USA* 99(20), 12889-12894 (October 1, 2002).

[27] Robert Lanza, "The troubling prospect of genetic manipulation" (Letter), *Washington Post*, p. A20, December 13, 2004. Curiously, in September 2002, Advanced Cell Technology, the company for which Dr. Lanza works, filed a patent for producing genetically altered artificial embryo-like entities, partly for the same purpose. The company's patent application summarizes the main idea: "Methods for making human ES cells and human differentiated cells and tissues for transplantation are described, whereby the cells and tissues are created following somatic cell nuclear transfer. The nuclear transfer donor is genetically modified prior to nuclear transfer such that cells of at least one developmental lineage are de-differentiated, that is, unable to develop, thereby resolving the ethical dilemmas involved in reprogramming somatic cells back to the embryonic stage." (United States Patent Application 20020132346.)

[28] See, Melton, D. A., Daley, G. Q., Jennings, C. G., "Altered nuclear transfer in stem-cell research—a flawed proposal," *New England Journal of Medicine* 351, 2791 (2004).

[29] Rogers, N. T., et al., "Phospholipase C{zeta} causes Ca2+ oscillations and parthenogenetic activation of human oocytes," *Reproduction* 128(6), 697-702 (2004). See also Lin, H., et al., "Multilineage potential of homozygous stem cells derived from metaphase II oocytes," *Stem Cells* 21, 152-161 (2003).

[30] While it has not yet been demonstrated using humans oocytes, José Cibelli and coworkers have recently succeeded in obtaining a line of pluripotent stem cells from parthenogenetically activated eggs of the long-tailed macaque (a nonhuman primate). See Cibelli, J. B., et al., "Parthenogenetic stem cells in nonhuman primates," *Science* 295, 819 (2002).

[31] See, Kiessling, A. A., "Eggs Alone—Human parthenotes: an ethical source of stem cells for therapies?" *Nature* 434, 145 (2005).

[32] Brockes, J. P., "Amphibian limb regeneration: rebuilding a complex structure," *Science* 276, 81-87 (1997).

[33] See, for example, Chen, S., et al., "Dedifferentiation of lineage-committed cells by a small molecule," *Journal of the American Chemical Society* 126(2), 410-411 (2004); Odelberg, S. J., et al., "Dedifferentiation of mammalian myotubes induced by msx1," *Cell* 103(7), 1099-1109 (2000); and McGann, C. J., Odelberg, S. J., Keating, M. T., "Mammalian myotube dedifferentiation induced by newt regeneration extract," *Proceedings of the National Academy of Sciences USA* 98(24), 13699-13704 (November 20, 2001).

[34] The best known research is by Catherine Verfaillie and coworkers at the Stem Cell Institute at the University of Minnesota Medical School. See, for a recent update, Dr. Verfaillie's review essay on "Multipotent Adult Progenitor Cells" (MAPCs), published as Appendix J in *Monitoring Stem Cell Research*: *A Report of the President's Council on Bioethics*, January 2004 (also available online at www.bioethics.gov). A more recent publication, by scientists at the University of Miami in collaboration with French scientists, reports the cultivation of pluripotent human cells from bone marrow (of human adults and children), after a unique expansion and selection procedure under conditions that were designed to mimic the in vivo environment of the early human embryo. According to the report, these cells can be grown for extended periods of time in culture, *express genes similar to those expressed by embryonic stem cells in culture*, and can be stimulated to differentiate into various adult tissue types, including neuronal and pancreatic islet tissue. See D'Ippolito, G., et al., "Marrow-isolated adult multilineage inducible (MIAMI) cells, a unique population of postnatal young and old human cells with extensive expansion and differentiation potential," *Journal of Cell Science* 117, 2971-2981 (2004). The relation between the MIAMI cells and Dr. Verfaillie's MAPCs is unclear, although it is possible that the MIAMI cells may be somewhat less differentiated than the MAPCs. The research from both laboratories needs to be reproduced by other scientists before its true promise can be assessed. See also Yoon, Y-S., et al., "Clonally expanded novel multipotent stem cells from human bone marrow regenerate myocardium after myocardial infarction," *The Journal of Clinical Investigation* 115, 326-338 (2005), and Kogler, G., et al., "A new human

somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential," *Journal of Experimental Medicine* 200, 123-135 (2004).

[35] See, in particular, Byrne, J. A., et al., "Nuclei of adult mammalian somatic cells are directly reprogrammed to oct-4 stem cell gene expression by amphibian oocytes," *Current Biology* 13, 1206-1213 (2003), and Gurdon, J. B., Byrne, J. A., Simonsson, S., "Nuclear reprogramming and stem cell creation," *Proceedings of the National Academy of Sciences USA* 100 (Suppl. 1), 11819-11822 (September 30, 2003).

[36] For the Council's discussion that gave rise to these provisional conclusions, see transcript of the Council's March 4, 2005, meeting (session 5), available online at www.bioethics.gov.

APPENDIX

Personal Statements

The preceding text constitutes the official body of this White Paper; it stands as the work of the entire Council. In the interest of contributing further to public discussion of the issues, and of enabling individual members of the Council to speak in their own voice on one or another aspect of this White Paper, we offer in this Appendix personal statements from those members who have elected to submit them:

Personal Statement of Dr. Gazzaniga

I do not support publishing this report with the implied endorsement that special efforts be made in the scientific areas described. While some of the suggestions could be explored in a scientific setting, most are high-risk options that only have an outside chance of success and raise their own complex set of ethical questions. Of primary concern: this effort is a diversion from the simple task at hand which is to move forward with the established laboratory techniques, that are already grounded on a clear ethical basis, for studying embryonic stem cell research and biomedical cloning.

The simple proposals that are now widely accepted by the majority of ethicists and scientists alike are as follows:

Allow the use of spare IVF embryos to develop more human stem cell lines. These are entities that do not possess a single neuron and are ready to go and can create tens of thousands of cell lines. Put another way, a piece of DNA is not a human being. A human being is an entity with a functioning brain consisting of billions of neurons with trillions of synapses that develops over time and with crucial interactions with the environment.

Allow biomedical cloning (SCNT) to go forward. This laboratory procedure has been tested and it works. SCNT can only be carried out in a laboratory and the 14-day-old entity that results from the procedure also has not a single neuron. After the specific stem cells are harvested by 14 days, the remaining tissue is disposed of.

From a purely scientific point of view I offer the following remarks on the alternative suggestions made by the Council, based on consultation with stem cell experts:

Proposal 1, the Landry-Zucker proposal, is absolutely dependent on defining a battery of "death markers." The criteria they offer lack the specificity and selectivity required. To what degree, for example is "embryo-dead" analogous to "brain dead?" What are the long-term

consequences (through adulthood) of using blastomeres from "dead" morulae? These are answerable, but formidable problems. Blastomere removal must be validated in vitro and in vivo, with respect to mitosis, survival, differentiation, etc., none of which are inaccessible. No assumption whatsoever can be made that these are "normal cells." At the same time, these questions are scientifically accessible.

Proposal 2, relies on biopsy, and this represents a new field, which must be explored experimentally. For example, and most obviously, what is the relationship of cellular to organ removal and transplantation? Are the biological rules homologous? We need the data.

Proposal 3, Bill Hurlbut's proposal, is also scientifically tractable, though fraught with ethical conundrums. Defective nuclear transfer could certainly be accomplished in theory, though issues of viability, etc., would be paramount. And replacing genes at the "wrong" time could be devastating either acutely or chronically. Although a myriad of additional potential problems could be cited, they are again empirical, not theoretical. Why delay what we know works with this sideshow? As pointed out in the paper, one of the leading stem cell research scientists in the world, Dr. Doug Melton at Harvard, has written an article in the *New England Journal of Medicine* dismissing this idea both scientifically and practically.

Proposal 4, dedifferentiation, is similarly approachable scientifically, though it is a high-risk strategy. Questions, however, raised with this idea can be answered with a huge and costly scientific program in place.

At the same time and from an ethical perspective, I must add that I find this proposal strained at best. Winding the clock back on a developed somatic cell and to stop it at a critical point is supposed to be void of ethical issues while letting a cell grow forward to just before the same point as with SCNT is not ethical?

This is the potentiality argument in reverse. In one version the film of life is running forward and in the other it is running in reverse. In both scenarios humans are making decisions about life and its origins.

It reveals how the so-called ethical concerns are being laid over molecular events moving either forward or backward in time.

In summary, these proposals, in spite of being experimentally approachable, are, ultimately, high-risk gambles. Each presents formidable, but not impossible, technical problems. The scientific challenges must not be confounded with the complex moral issues. Most troubling, this effort dilutes the essential question: Is the United States of America going to allow embryonic stem cell research and biomedical cloning to go forward using the now widely accepted techniques used by the private sector, by the State of California, and by dozens of other countries, or is it going to remain hostage to the arbitrary views of those with certain beliefs about the nature of life and its origins?

MICHAEL S. GAZZANIGA

Personal Statement of Professor George
(joined by Professor Glendon and Dr. Gómez-Lobo)

I support the Council's efforts to identify means of obtaining human pluripotent stem cells for biomedical research that do not involve killing or harming human embryos and do not invite the exploitation of women to obtain ova. If such means can be identified, research involving embryonic or embryonic-type stem cells could go forward, and be funded by the federal government, without ethical qualms and controversy. Assuming that supporters of embryonic stem cell research and its public funding are sincere in saying that they have no intention or desire to derive tissue or organs from post-implantation human embryos or from human fetuses, this would bring to a close—honorably and without rancor—a divisive chapter in our recent history. Frankly, I do not see how any person of goodwill could be opposed to such a resolution of the matter.

I commend everyone who has stepped forward to propose possible methods of obtaining human pluripotent stem cells while fully respecting human life at every stage of development. I thank our Council's staff and consultants who have helped to provide in this White Paper a thorough, if necessarily in some ways still preliminary, analysis of each of the four proposals we sketch and analyze. Although I do not hold out hope of obtaining pluripotent stem cells harmlessly via blastomere extraction from living human embryos (proposal II), I believe that each of the other three proposals merits further exploration (including, where indicated, experimental research involving non-human animal cells) and analysis.

The best long-term solution is likely to be somatic cell dedifferentiation (proposal IV). But while scientists work towards the goal of dedifferentiating somatic cells back to their corresponding progenitor cells, it *may be* possible ethically to employ one or both of the other two methods, namely deriving cells from embryos that have died (proposal I) or from nonembryonic entities produced by altered nuclear transfer (proposal III). I say, with emphasis, *may be* because serious moral and practical concerns have been raised about both

proposals. Research and further analysis will be required to determine whether the proposed methods are technologically possible and ethically sound. That research and analysis should, in my opinion, go forward.

Of the four possible methods explored in our White Paper, the one that has attracted the most intense interest outside the Council is altered nuclear transfer. There are two major concerns: (1) the question whether the entity produced would be truly non-embryonic, and not a disabled embryo or an embryo genetically programmed for a premature death; and (2) the question whether ova could be supplied without subjecting women to the painful and possibly dangerous process of superovulation. Neither of these questions is, strictly speaking, ethical, though both have what I consider to be decisive ethical implications. Like Dr. Hurlbut, who has taken the lead in formulating this proposal, I will not support altered nuclear transfer as a method of obtaining human pluripotent stem cells unless it can be shown that (1) the procedure truly and reliably produces nonembryonic entities, rather than damaged embryos, and (2) it is possible to carry out altered nuclear transfer on the scale required without subjecting women to harmful and exploitative practices.

I recognize that some people have objections to altered nuclear transfer even if these conditions are met. Dr. Krauthammer, for example, objects even if the sources of stem cells created can be shown truly to be nonembryonic. Because Dr. Krauthammer also objects (as I do) to the creation for destruction of true embryos (by cloning or any other method), I take his concerns very seriously and welcome his criticisms of my own more permissive view. I would not finally endorse altered nuclear transfer using human cells prior to engaging the argument with him more fully and considering with the utmost care the considerations he adduces against it.

It is more difficult to credit the ethical objections to altered nuclear transfer of those who support the creation of true embryos to be destroyed in biomedical research. How can it be right deliberately to create and destroy true human embryos—beings that no one can deny are human individuals in the embryonic stage of development—yet somehow wrong to produce disorganized growths that are the moral equivalent of gamete tumors rather than embryos?

One final point: the effort in which I am happy to join to find morally legitimate means of obtaining embryonic or embryonic-type stem cells should not be interpreted as indicating any acceptance of the hyping of the therapeutic promise of embryonic stem cell research that has marred the debate over the past four years. This promotion of exaggerated expectations dishonors science and shames those responsible for it by cruelly elevating the hopes of suffering people and members of their families. It should be condemned.

ROBERT P. GEORGE
(JOINED BY MARY ANN GLENDON
AND ALFONSO GÓMEZ-LOBO)

Personal Statement of Dr. Hurlbut

Since I find myself in the unusual position of being both a member of the Council and, at the same time, the principal advocate of one of the four proposals here under consideration, I am mindful that a delicate balancing of roles is required in the way I frame the following remarks. I shall take care that my comments not cross the line separating impartial analysis of the four proposals from partisan advocacy of one of them.

While I believe that, by and large, the White Paper, like a good preliminary hearing at law, does an admirable job of presenting the four proposals and beginning their ethical, scientific, and practical analysis, it seems to me deficient in two main respects. First, I think there is much more to be said about the importance, for the future, of finding a scientific and ethical solution to the problem of deriving human stem cell lines. Second, I think that some of the ethical concerns raised about the ANT proposal could have been answered more effectively. In the brief remarks that follow, I will try to supply these two deficiencies.

1. General comments about the importance of the project

In our report *Human Cloning and Human Dignity: An Ethical Inquiry* (July 2002), I joined colleagues who called for a moratorium on cloning-for-biomedical-research. I believed that our nation needed time to consider more thoroughly the moral status of ex vivo human embryos and to seek scientific methods for procuring embryonic stem cells that could sustain social consensus for a unified federal policy. This White Paper both deepens the dialogue and encourages progress toward these goals, but it does not make it sufficiently clear why it is urgent that we succeed.

The past three years have been characterized by controversy about the scientific prospects for embryonic (versus adult) stem cells and a widening political divide over the ethics of ESC research. Advances

have been made in the study of ES cells, yet predictions of imminent cures have been moderated by a recognition of the technical difficulties in emulating in vitro the intercellular signals and microenvironments that promote cell differentiation within natural embryogenesis. Nonetheless, scientists generally believe that ESC research is both essential to the broader study of both natural development and pathogenesis and promising for medical interventions against a range of serious diseases. They also believe that, without NIH support for newly created ES cell lines, progress in this important realm of research will be severely constrained.

Yet even as these scientific prospects have become clearer, advances in our understanding of developmental biology have strengthened the case of those with ethical objections to embryo destruction. New scientific evidence supports the idea that there is an integrated unity and unbroken continuity of development from fertilization onward—and undercuts claims that the early embryo is an "inchoate clump of cells," available for instrumental use with little or no moral concern.

These findings have solidified the convictions of many people that any instrumental use of human embryos must be acknowledged as a choice for destruction of human life (albeit at a very early stage of development), justified not by scientific evidence or moral reason but by a purely utilitarian calculus based on the promise of cures and even commercial considerations. This approach, grounded not on principle but on a balance of benefits, would seem vulnerable to being easily persuaded toward additional exceptions and extensions as further promising projects become evident.

Beyond their destruction for the procurement of ES cells, some fear the industrial scale production of living human embryos for a wide range of research in natural development, toxicology, and drug testing. These concerns have already been aggravated by proposals to use ES cells in the creation of human-animal chimeras, including projects involving their gestation to various stages of development in the wombs of animals. Furthermore, some see ominous implication in the evidence that later stage human embryos (beyond 14 days) can provide

the critical conditioning environment for the transformation of adult stem cells into useful cell types, tissues, and possibly organs.*

Our current conflict over the moral status of the human embryo reflects deep differences in our basic convictions and is unlikely to be resolved through deliberation or debate. Likewise, a purely political solution will leave our country bitterly divided, eroding the social support and sense of noble purpose that is essential for the public funding of biomedical science. Furthermore, the emerging patchwork of state policies threatens to create a situation in which many patients will enter the hospital with qualms about the moral foundations on which their treatments have been developed. The traditional sanctuary of compassionate care at the most vulnerable and sensitive moments of human life is becoming an arena of controversy and conflict.

As we enter the era of developmental biology, we will face many more moral dilemmas; the current conflict over ES cells is just the first of a series of difficult controversies over the experimental use of emerging life that will require us to define with clarity and precision exactly which boundaries we seek to defend.† Chimeras, parthenotes, and projects involving the reaggregation of ES cell products will continue to challenge our definitions of human life. *How we act now in the stem cell dilemma will set a precedent for all future efforts to exploit nascent human life for scientific ends*. There is thus much more at stake than the proposals herein discussed.

The proposals presented in this White Paper open a realm of intellectual inquiry and creative scientific investigation in the search for

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* See Yokoo, T., et al., "Human mesenchymal stem cells in rodent whole-embryo culture are reprogrammed to contribute to kidney tissue," *Proceedings of the National Academy of Sciences USA* 102(9), 3296-3300 (March 1, 2005).

† These studies will not be limited to ES cells and the first few days of embryogenesis, since there are compelling scientific and medical reasons to seek an understanding of the entire trajectory of human biology from fertilization to natural death. Beyond the obvious benefit of understanding the fundamental biological factors behind the estimated 200,000 birth defects per year, it is becoming increasingly evident that pathologies that manifest themselves in adult life (such as hypertension, diabetes, etc.) are influenced by, or have their origins in, early development.

a solution to our current impasse over the procurement of embryonic stem cells. Such a solution must be grounded in deep ethical reflection and in careful preliminary studies with animal cells. The incommensurate good of human life, and the corresponding danger of its instrumental use (thereby violating the principle we are trying to protect), mean that the highest levels of caution must prevail as we proceed forward with this project. We must initiate the cooperative dialogue that is essential to frame the moral principles that can at once defend human dignity and promote the fullest prospects for scientific progress and its medical applications.

2. Answers to ethical concerns raised about ANT

Throughout this report we draw a distinction between *pluripotency*, the capacity to give rise to many if not all the different cell types of the human body, and *totipotency*, the capacity to give rise to the whole organism as an integrated living being. Employing these concepts in the search for a technological solution to our ethical impasse, we must consider any entity that has the intrinsic potential to develop as a human organism (totipotency) as bearing the inviolability of a human life.* This status applies regardless of its means of production or present location.

Proposals 1 (Landry-Zucker) and 3 (Altered Nuclear Transfer) may hold the best near-term promise for practical application, yet they also raise the most difficult conceptual considerations. Landry-Zucker would extract ES cells from embryos that are no longer totipotent, and Altered Nuclear Transfer (ANT) would create and extract ES cells from "biological artifacts" that never rise to the level of totipotency. Both proposals shift the ethical debate from the question of *when* a normal embryo is a human being with moral worth, to the more fundamental question of *what* component parts and organized structure constitute the minimal criteria for considering an entity a living human organism.

Each of these proposals draws on the idea that a living organism is a self-subsisting being, a coordinated and coherent whole with the

* A practical measure of this intrinsic potential would be the ability to develop when provided the support and nurture of a natural gestational environment or its technological equivalent.

capacity for self-directed development, maintenance, and repair. The very word *organism* implies organization, an overarching principle of unity, a cooperative interaction of interdependent parts subordinated to the good of the whole. For an embryonic organism, this implies an inherent potency, a drive in the direction of the mature form. By its very nature, an embryo is a developing being, its wholeness defined by both its manifest expression and its latent potential; it is the phase of human life in which the organismal whole produces its organic parts.

For Landry-Zucker, the conceptual and moral challenge is to define the meaning of "embryo death," the cessation of integrated form and the totipotent capability that characterizes a living human embryo. A secondary, scientific challenge is to identify a physical marker of this state. For Altered Nuclear Transfer, the conceptual and moral challenge is the more difficult task of defining the boundary between mere cellular growth lacking integrated form and a living organism. The scientific challenge of ANT is to find the right genetic or epigenetic alteration to ensure that pluripotent cells can be produced while not creating an embryonic human being. It is here that the White Paper, in my view, does not make sufficiently clear how the proposal in fact meets that challenge.

That an ANT product might during its earliest stages visually resemble an embryo does not make it an embryo, for an entity's fundamental nature must ultimately be based on its internal biochemical structure and organization. Likewise, cell division and growth are not sufficient evidence that the entity is a human embryo. Even an egg without a nucleus, when artificially activated, has the developmental power to divide to the eight-cell stage, yet clearly is not an embryo, or even an organism. Moreover, the possibility that the alteration could be reversed does not affect the fact that the targeted alteration has preempted the ANT entity from having the nature (the ontological status) of an embryo.

The product of ANT would, by intention, lack the active potential and inviolable moral nature of a living human being. Without this moral standing, there is no obligation of repair because there is no living being to be repaired. Nonetheless, even such a limited biological entity should be accorded a certain cautionary respect as with all human tissues, though not the full protection of human life.

Some fear that the precedent of intentional genetic intervention (essential to ANT), and its justifying argument based on the intrinsic insufficiency of the entity produced, could become the basis for further projects in the bioengineering of ever more human-like "intermediate biological forms." This is a serious consideration, but one that would be better addressed to those who maintain an "intermediate moral status" (worthy of dignity but not inviolability) for the human embryo and already accept the destruction of fully normal human beings at an early stage of their development.

The very foundation of the moral argument for ANT should work to mitigate the concerns about the "slippery slope" potential for ES cell research. Since ANT seeks to defend human dignity from conception, it is less likely to lead to such indiscriminate and instrumental use of human life than the practices it seeks to preempt. By establishing a principled concern for the protection of human life from fertilization to natural death, ANT sets a firm foundation for the later distinctions necessary for further moral discrimination. Other proposals for the procurement of ES cells (SCNT and "leftover" IVF embryos) give little or no guidance to override the persuasive power of further promise from extending exceptions to moral principles. By establishing the primacy of ethical principle as the foundation of all scientific progress, ANT could help set the foundation and frame for the additional ethical dilemmas that will inevitably arise with advances in developmental biology. The difficult definitions and distinctions established in the moral deliberations associated with the ANT proposal could help chart the course and protect the path of future projects in this emerging arena of biology. If slippery slope arguments express prudential concerns, it seems reasonable to weigh ANT against the much more slippery scenario that will likely follow in its absence.

Finally, there is the less easily argued but wider wisdom of our intuitive aesthetic response, and the concern that somehow we may violate or distort the principles of natural order that sustain the coherence and sense of significance of human life. I consider this the most compelling objection to both ANT and the whole of the modern project of biological intervention in the natural world. Clearly, no project that enters into such proximity with the most central and sacred realms of human life should be undertaken without a sense of

cautionary concern and serious purpose. Employing these powers of our most basic biology demands a sensitive awareness of the radiance of respect that must attend any technological use of body parts or processes apart from their proper place in the larger purposes of life. Nonetheless, where great good is possible, human tissues and organs have been used in the service of healing, and in this coming era of control over the primary forces of developmental biology, we will learn to use partial trajectories of organic growth even apart from their context within the living whole of the human body. The moral concerns and sensitivities that animate the proposal for ANT can, in fact, enable us to do so without losing our humanity.

WILLIAM B. HURLBUT

Personal Statement of Dr. Rowley

Proposal 1

Landry and Zucker propose to thaw out embryos to follow the natural history of "dead" embryos. Because they do not know in advance which embryos will not divide and which will, some portion of embryos (about half) will continue to divide and will be healthy embryos. What happens to these healthy embryos? The proposal says healthy embryos in excess of those to be implanted will be allowed to die while scientists struggle to recoup a few living cells from the dead embryos! This seems to me to be the height of folly. As noted on page 21 in a footnote, I raised this concern during the public discussion.

Proposal II

I think this is risky research, although I recognize it is currently done as part of prenatal genetic diagnosis. In the latter case, it is done to prevent implantation of an embryo with a serious disease present in the parents; in the former, in the present proposal, it is done to circumvent a problem that causes ethical concerns to some people. There are at least two critical questions: can you get a cell line from one cell or two, and does it harm the embryo that will subsequently be implanted? Just because experiments in mice seem to indicate that it is feasible in mice (but with increased inter-uterine mortality), does not mean it will work for human embryos. In support of this proposal, it is absurd to say that this cell line, if it grows, will be a source of cells for the child later in life. A much more effective procedure would be to harvest stem cells from the cord blood and preserve them.

Proposal III

This proposal is scientifically unsound, and for individuals concerned about manipulating human oocytes for experiments, it should be ethically unacceptable! It has proven very inefficient to remove the nucleus from a human oocyte and to replace it with a

normal, unmanipulated nucleus from a donor. In the proposed experiments, the donated nucleus will be made defective by some uncertain genetic strategy. The defective nucleus will be inserted into the enucleated oocytes, the oocytes will be stimulated to grow, and then the genetic defect will be corrected so that the cells are "normal." In my view, this research asks women to donate oocytes for research that is highly unlikely to result in cell lines that would be useful for treating sick patients, which is the purpose of trying to perfect the development of human embryonic stem cell lines.

Proposal IV

This proposal does not involve embryos, but rather differentiated cells. The purpose aims to dedifferentiate these cells. At least some of the purported successes with this strategy have had flaws when examined carefully. This proposal should be submitted to the National Institutes of Health and if it passes peer review with a sufficiently high score to be funded, then the research will go forward.

My concerns with many of these proposals is that they will use financial resources that would be better devoted to proposals that are likely to be more productive. I find the notion that it is ethically sound to let healthy embryos die rather than use them to try to develop cell lines that could benefit sick and dying patients totally baffling. We talk about protecting human dignity. We should strive to help patients with serious illnesses that could potentially be treated with embryonic stem cells to live as fulfilling and dignified lives as is humanly possible. The research proposed in this White Paper largely fails to achieve this good, and thus I cannot support proposals I, II and III.

JANET D. ROWLEY

Personal Statement of Professor Sandel

I share the goal of seeking ethically uncontroversial ways of pursuing embryonic stem cell research. In my view, the first proposal (deriving cells from dead embryos) and the fourth (somatic cell dedifferentiation) are ethically acceptable and worthy of further exploration. I find the third proposal (deriving cells from specially engineered biological artifacts) to be morally objectionable.

As one who supports embryonic stem cell research, I do not regard the early embryo as inviolable. But neither do I regard it as disposable, open to any use we may desire or devise. For this reason, embryo research carries a special moral burden; it is justified only for the sake of saving human lives or curing devastating diseases. The proposal to genetically engineer a nonviable, embryo-like being would remove the moral burden by creating something that, lacking the capacity to develop into a human person, would be wholly disposable, presumably for any purpose, weighty or trivial. The very project of creating such a being is morally troubling, for reasons that are well-stated in the ethical analysis (pp. 38-45 above). I therefore do not believe that this proposal should be encouraged or endorsed.

MICHAEL J. SANDEL

Glossary*

Adult stem cell: An undifferentiated cell found in a differentiated tissue that can renew itself and (with certain limitations) differentiate to yield all the specialized cell types of the tissue from which it originated. (NIH)

Altered Nuclear Transfer (ANT): A proposed method, using a modified form of somatic cell nuclear transfer (SCNT), of producing a biological artifact from which human pluripotent stem cells could be derived.

Anencephalic fetus: A fetus with a congenital defect related to development of the brain, with absence of the bones of the cranial vault and absent or rudimentary cerebral and cerebellar hemispheres, brainstem, and basal ganglia. (SMD)

Aneuploid: Having an abnormal number of chromosomes. (SMD)

Autologous: Derived or transferred from the same individual's body.

Biological artifact: As employed here, this phrase denotes an artificially created non-embryonic but embryo-like cellular system, engineered to lack the essential elements of embryogenesis but still capable of some cell division and growth.

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* Definitions marked "(CR)" are from the Council's report on human cloning (*Human Cloning and Human Dignity: An Ethical Inquiry*, Washington, D.C.: Government Printing Office, 2002). Definitions marked "(NIH)" are from the National Institutes of Health online stem cell glossary at http://stemcells.nih.gov (accessed April 1, 2005). Definitions marked "(NRC)" are from the National Research Council report, *Stem Cell Research and the Future of Regenerative Medicine* (Washington, D.C.: National Research Council, 2001). Definitions marked "(SMD)" are from Stedman's Medical Dictionary.

Biopsy: Process of removing tissue from patients for diagnostic examination. (SMD)

Blastocyst: In mammals, an early stage of embryonic development at which the embryo (roughly 100-200 cells) is a hollow sphere made up of an outer layer of cells (the trophectoderm), a fluid-filled cavity (the blastocoel), and a cluster of cells on the interior (the inner cell mass).

Blastomere: A cell contained within an early embryo (up to two days after conception, at which point the embryo comprises about 8 blastomeres).

Blastomere biopsy: Removal of one or two blastomeres from the embryo in vitro at about the 8-cell stage, usually in order to perform preimplantation genetic diagnosis and screening.

Blastula: An early stage of embryonic development (roughly 100-200 cells) at which the cells of the morula are rearranged to form a hollow sphere; at this stage of embryonic development in humans and other mammals, the embryo is generally called a *blastocyst*.

Bone marrow: The soft, fatty, vascular tissue that fills most bone cavities and is the source of red blood cells and many white blood cells.

Chimera: In experimental embryology, the individual produced by grafting an embryonic part of one animal on to the embryo of another, either of the same or of another species. (SMD)

Chromosomes: Structures inside the nucleus of a cell, made up of long pieces of DNA coated with specialized cell proteins, which are duplicated at each mitotic cell division. Chromosomes thus transmit the genes of the organism from one generation to the next. (CR)

Cleavage arrest: Spontaneous cessation of cell division in an early embryo.

Cloned embryo: An embryo arising from the somatic cell nuclear transfer process as contrasted with an embryo arising from the union of an egg and sperm. (CR)

Cloning:

Cloning-to-produce-children—Production of a cloned human embryo, formed for the (proximate) purpose of initiating a pregnancy, with the (ultimate) goal of producing a child who will be genetically virtually identical to a currently existing or previously existing individual.

Cloning-for-biomedical-research—Production of a cloned human embryo, formed for the (proximate) purpose of using it in research or for extracting its stem cells, with the (ultimate) goals of gaining scientific knowledge of normal and abnormal development and of developing cures for human diseases.

Human cloning—The asexual reproduction of a new human organism that is, at all stages of development, genetically virtually identical to a currently existing, or previously existing, human being. (CR)

Cord blood: Blood in the umbilical cord and placenta.

Cryopreservation and **Cryostorage:** Freezing of IVF embryos for later use.

Cytoplasmic: Of or pertaining to the substance of a cell, exclusive of the nucleus. (SMD)

Dedifferentiation: A procedure whereby differentiated, somatic cells are restored to a more undifferentiated, multipotent condition.

Diploid: Refers to the full complement of chromosomes in a somatic cell, distinct for each species (forty-six in human beings). (CR)

Embryo: (a) In humans, the developing organism from the time of fertilization until the end of the eighth week of gestation, when it becomes known as a fetus. (NIH) (b) The developing organism from the time of fertilization until significant differentiation has occurred, when the organism becomes known as a fetus. An organism in the early stages of development. (CR)

Embryogenesis: That phase of prenatal development involved in establishment of the characteristic configuration of the body of the

embryo; in humans, embryogenesis is usually regarded as extending from the end of the second week to the end of the eighth week, after which the product of conception is usually spoken of as a fetus. (Based on SMD)

Embryonic germ layers: The three initial tissue layers arising in the embryo—endoderm, mesoderm, and ectoderm—from which all other somatic tissue-types develop. (NRC)

Embryonic stem cells (ESCs): Primitive (undifferentiated) cells, derived from the inner cell mass of the embryo, that have the potential to become a wide variety of specialized cell types. (Based on NIH)

Enucleated oocyte: An egg cell from which the nucleus has been surgically removed.

Ex vivo: Outside the body, frequently the equivalent of "in vitro"; the opposite of "in vivo."

Fertilization: The process whereby male and female gametes unite. (NIH)

Fetus: A developing human from usually two months after conception to birth. (NIH)

Gamete: A reproductive cell (egg or sperm). (CR)

Gene: A functional unit of heredity that is a segment of DNA located in a specific site on a chromosome. A gene directs the formation of an enzyme or other protein. (NIH)

Genome: The total gene complement of a set of chromosomes. (SMD)

Genotype: The genetic constitution of an organism or a group of organisms. (SMD)

Hydatidiform mole: An abnormality during pregnancy; a tissue mass or growth that forms within the uterus as the result of a genetic error during the fertilization process.

Implantation: The attachment of the blastocyst to the lining of the uterus, and its subsequent embedding there. (Based on SMD)

In vitro fertilization (IVF): The union of an egg and sperm, where the event takes place outside the body and in an artificial environment (the literal meaning of "in vitro" is "in glass"; for example, in a test tube). (CR)

Inner cell mass: The cluster of cells inside the blastocyst. These cells give rise to the embryonic disk of the later embryo and, ultimately, the fetus. (NIH)

IVF embryo: An embryo produced by in vitro fertilization.

Karyotype: The chromosome characteristics (number, shape, etc.) of an individual cell or cell line, usually presented as a systematized array in pairs. (SMD)

Lineage: The descendants of a common ancestor.

Mesenchymal stem cells: Cells from the immature embryonic connective tissue. A number of cell types come from mesenchymal stem cells, including chondrocytes, which produce cartilage. (NIH)

Morphology: Configuration or structure, shape.

Morula: An early stage of embryonic development (roughly 16-64 cells) at which the embryo is a solid spherical mass of cells, resulting from the early cleavage divisions of the zygote; so called because of its resemblance to a "little mulberry" (in Latin, *morula*).

Mosaic: Possessing two or more genetically different cell types; an early embryo is said to be *mosaic* when some of its cells exhibit chromosomal abnormalities while others appear chromosomally normal.

Multipotent adult progenitor cells (MAPCs): Cells isolated from bone marrow that can be differentiated into cells with characteristics of cartilage, fat, and bone.

Multipotent cell: A cell that can produce two or more different types of differentiated cells; adult stem cells are *multipotent*.

Oocyte: Unfertilized egg cell.

Organismic death (of an embryo)—concept and **criterion:** As proposed by Landry and Zucker, the *concept* of *organismic death* for an early-stage human embryo is defined by irreversible loss of "the capacity for continued and integrated cellular division, growth, and differentiation"; their proposed *criterion* for determining organismic death is "irreversible cessation of cell division in the embryo observed in vitro."

Parthenogenesis: A form of reproduction in which an unfertilized egg develops into a new individual (SMD); the process of inducing an unfertilized egg to initiate cell division.

Parthenote: The primary product of parthenogenesis; more precisely, an unfertilized egg that has been activated to initiate cell division.

Placenta: The oval or discoid spongy structure in the uterus from which the fetus derives it nourishment and oxygen. (NRC)

Pluripotent cell: A cell that can produce all the cell types of the developing body; embryonic stem cells, as well as the inner cell mass cells of the blastocyst, are *pluripotent*.

Pluripotent stem cell: Any stem cell that has the same *functional capacity*—that is, stable pluripotency—as an embryonic stem cell, though not necessarily the same *origin*.

Preimplantation genetic diagnosis (PGD): A method of testing IVF embryos for chromosomal or genetic disorders before they are transferred to the uterus; typically one or two blastomeres are removed for genetic testing at about the 8-cell stage of embryonic development.

Somatic cell: Any cell of an organism other than the gametes. (Based on SMD)

Somatic cell nuclear transfer (SCNT): A method of cloning: transfer of the nucleus from a donor somatic cell into an enucleated oocyte to produce a cloned embryo.

Stem cells: Stem cells are undifferentiated multipotent precursor cells that are capable both of perpetuating themselves as stem cells and of undergoing differentiation into one or more specialized types of cells. (CR)

Stem cell line: Stem cells which have been cultured under in vitro conditions that allow proliferation without differentiation for months to years. (NIH)

Superovulation: Drug-induced stimulation of a woman's ovaries to produce many mature oocytes in a single menstrual cycle.

Teratoma: A tumor consisting of different types of tissue, as of skin, hair, and muscle, caused by the development of independent germ cells. (SMD)

Totipotent cell: A cell that can give rise to the entire organism, including the extra-embryonic membranes; the fertilized egg or zygote is *totipotent*.

Trophectoderm: In early embryos at the blastocyst stage, the outer layer of cells that will give rise to the placenta.

Uterine transfer: Transfer of an IVF embryo to a woman's uterus with a view to implantation and gestation.

Xenotransplantation: A transplant of tissue from an animal of one species to an animal of another species.

Zygote: The diploid cell that results from the fertilization of an egg cell by a sperm cell. (CR)