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March 24, 2010

NIH Stem Cell Guidelines
MSC 7997
9000 Rockville Pike
Bethesda, Maryland, 20892-7997

Ladies and Gentlemen:

Genetics Policy Institute ("GPI") is pleased to respond to the request from the National Institutes of Health ("NIH") in its proposing release, dated February 16, 2009 (the "Proposing Release"), for comments on its proposed revision to the definition of human embryonic stem cells ("hESCs") in the National Institutes of Health Guidelines for Human Stem Cell Research (the "Guidelines"), released on July 6, 2009 (effective beginning July 7, 2009).

GPI is a not-for-profit organization formed in 2003 with the mission of promoting and defending stem cell research and its application in medicine to develop therapeutics and cures for many otherwise intractable diseases and disorders. GPI seeks to catalyze and help lead a global network of influential stakeholder groups representing patient advocates, scientists, physicians and health care professionals, industrialists, bioethicists, lawyers, educators, and policy-makers. GPI pursues this mission through cosponsorship and management of its flagship annual World Stem Cell Summit, publication of the World Stem Cell Report, special projects, speaking engagements, teaching initiatives, and strategic collaborations. Upon this base of activities and relationships, GPI serves as a communications channel, helping to build the knowledge base needed for ethical and thoughtful policy-making in support of scientifically and medically worthy research and clinical translation in the field of stem cells and the broader field of developmental biology.[1] For more information about GPI, please refer to our web site at www.genpol.org.

GPI fully recognizes the gravity of the ethical responsibilities entailed by research with hESCs and human induced pluripotent stem cells ("iPSCs"), as well as regenerative medicine arising from this research, and the need for the Guidelines to fully respect and reflect these responsibilities. We thus duly note NIH's comment in the Proposing Release that the "proposed change [in the definition of hESCs in the Guidelines] in no way alters the rigorous ethical

[1] In undertaking this role, GPI maintains and consults with its Board of Advisors. GPI extends it gratitude to the following members of this Board of Advisors for their assistance in preparing this letter: Prof. Janet L. Dolgin (Hofstra University School of Law); Alan L. Jakimo, Esq. (Partner, Sidley Austin, LLP; Special Professor of Law – Hofstra University School of Law); Rosario Isasi, J.D., M.P.H. (Research Associate at the Centre of Genomics and Policy, Faculty of Medicine, Dept. of Human Genetics at McGill University); and Prof. Russell Korobkin (University of California-Los Angeles School of Law).

standards set forth in the Guidelines." Given the ethical significance of this matter we believe that the text of the Guidelines must be as consistent as possible with the body of other documents and sources of information relating to stem cell research and regenerative medicine published and made accessible by NIH, including, for example and without limitation, the glossary of stem cell terms maintained by NIH as part of its web-based Stem Cell Information Center and accessible at http://stemcells.nih.gov/info/glossary.asp (the "NIH Stem Cell Glossary").

Summary of GPI's Comments

Our overarching comments, explained in detail below, distill to two points: ***first,*** we agree with NIH that the definition of hESCs should be revised to include stem cells obtained from embryos up to and including the blastocyst stage; but ***second,*** the definition of hESCs should not be limited to pluripotent cells.

Background

As stated in the Proposed Release, Section II of the Guidelines currently defines hESCs as "cells that are derived from the inner cell mass of blastocyst stage human embryos, are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers." The NIH Stem Cell Glossary also defines an hESC as "[a] type of pluripotent stem cell derived from the inner cell mass (ICM) of the blastocyst."

In the Proposing Release, NIH explains that this blastocyst-based definition excludes hESCs that are derived from embryos that fail to develop to the blastocyst stage, and that NIH did not intend in the Guidelines to exclude such hESCs from eligibility for federal funding.

Accordingly, NIH is proposing to revise the definition of hESCs to mean "pluripotent cells that are derived from early stage human embryos, up to and including the blastocyst stage, are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers."

This revised definition thus embodies two changes:

> *first,* insertion of the word "pluripotent" before the phrase "cells that are derived"; and
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> *second,* substitution of the phrase "from early stage human embryos, up to and including the blastocyst stage" for the phrase "from the inner cell mass of blastocyst stage human embryos".

In sum, GPI believes that the word "pluripotent" should not be inserted in the hESC definition and that the second change, without the phrase "early stage", should be made.

Proposed Revision #1: Insertion of the Word "Pluripotent"

For reasons we offer below, we believe that the word pluripotent should not be inserted in the definition. Notwithstanding this comment, if NIH determines to limit the definition of hESCs for purposes of the Guidelines to pluripotent cells, then revision must also be made to the phrase "are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers".

The NIH Stem Cell Glossary, in one sentence, defines the term "pluripotent" to mean "having the ability to give rise to all of the various cell types of the body", and follows that sentence with two explanatory points. In the first of these two points, NIH interprets the term "body" by explaining that "[p]luripotent cells cannot make extra-embryonic tissues such as the amnion, chorion, and other components of the placenta." In the second, NIH explains that "[s]cientists demonstrate pluripotency by providing evidence of stable developmental potential, even after prolonged culture, to form derivatives of all three embryonic germ layers from the progeny of a single cell and to generate a teratoma after injection into an immunosuppressed mouse."

If NIH means for this statement of demonstrability of pluripotency to constitute a functional definition of the term pluripotency, then three points follow:

> *first,* in the NIH Stem Cell Glossary, the definition of pluripotency needs to be so revised to indicate that the statement of the manner by which pluripotency is demonstrated is more than explanatory, but definitional;
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> *second,* in the hESC definition in the Guidelines, the teratoma test in the statement of demonstrability of pluripotency needs to be added as a third element to the phrase "are capable of dividing…, and are known to develop…"; and
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> *third,* in the hESC definition in the Guidelines, as so revised according to the second step above, there is no need to add the word "pluripotent", as it would be redundant with the three-element phrase "are capable of dividing…, are known to develop…, and to generate a teratoma…".

Alternatively, if NIH determines to add the word "pluripotent" to the definition of the hESC in the Guidelines and NIH makes no change to the definition of pluripotent in the NIH Stem Cell Glossary, then to be consistent with the latter definition and achieve some economy of words, the definition of hESC in the Guidelines should read as follows:

> "pluripotent cells that are derived from early stage human embryos, up to and including the blastocyst stage, where the term 'pluripotent' has the meaning provided in the NIH Stem Cell Glossary of stem cell terms"
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> OR
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> "pluripotent cells that are derived from early stage human embryos, up to and including the blastocyst stage, where the term 'pluripotent' means having the ability to give rise to all of the various cell types of the body (excluding the cell types comprising the placenta)"

Proposed Revision #2: "from early stage human embryos, up to and including the blastocyst stage"

Upon first read, this proposed substitution presents a simple technical correction that, as NIH states in the Proposing Release, more accurately sets forth its original intention in the draft of the Guidelines proposed for comment in April 2009 (the "Draft Guidelines"). This point is well taken and we could end here with the mathematician's end-of-proof phrase "*quod erat demonstrandum*".

But upon further review we believe that this proposed technical revision illustrates an important point in the evolution of guidelines in the field of stem cell research and the related set of topics that GPI sought to address in its comment letter to NIH, dated May 26, 2009 (the "2009 GPI Letter"), relating to the Draft Guidelines. In Part VI of that letter ("Issues of Drafting Precision, Interpretation, and Implementation"), GPI made the observations excerpted below *[with footnotes renumbered and Exhibit B to the 2009 GPI Letter excluded]*. Along one dimension, these observations underscore the bona fides of NIH's statement as to its original intent in the Draft Guidelines. And along another dimension, these observations suggest that NIH may want to do more here to achieve a requisite level of precision and interpretability as it seeks to clarify the intended meaning of hESCs for purposes of the Guidelines.

> As a starting point, we urge NIH to include a glossary in the Final Guidelines. Inclusion of a glossary would follow the usual and customary convention of setting forth definitions for terms used in rules and guidelines promulgated by an agency of the executive branch of government. As the character of regulations and guidelines most often depends significantly on the specific definitions ascribed to terms of art therein, without a glossary in the Final Guidelines or Adopting Release, NIH risks substantive uncertainties in applying the Final Guidelines and the increased administrative expense entailed by the need to address these uncertainties on a case-by-case basis. In Exhibit B to this letter we present a list of several terms or their correlatives that appear in the Draft Guidelines and the Proposing Release for which definitions may prove helpful. *[Exhibit B intentionally excluded]*
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> A key term in the Final Guidelines, of course, will be the definition of "human embryonic stem cells". As described in the Draft Guidelines, "human embryonic stem cells are cells that are derived from human embryos, are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers." In its 2008 Guidelines, NAS [the National Academy of Sciences] defines the term "embryonic stem (ES) cells" as "primitive (undifferentiated) cells derived from the early embryo that have the potential to become a wide variety of specialized cell types." While there is a high degree of correspondence between these two definitions, the NAS definition includes a time component in referring to the "early embryo". This time component takes on significance when one realizes that the definition of "embryo" in scientific terms and the definition of "fetus" set forth in the Common Rule have a time overlap.[2] The NIH's draft guidelines in 2000 contained the word "early", but NIH deleted the word from the final 2000 guidelines noting in the preamble to those guidelines that "the Guidelines make it clear that NIH funding of research using hPSCs derived in the private sector from human embryos can involve only embryos that have not reached the stage at which the mesoderm is formed." Instead of a mesoderm-based reference, the Draft Guidelines uses an even more subtle approach in the phrase "known to develop into cells and tissues of the three primary germ layers." We believe that this may be too subtle for the lay reader, and that also using the term "early embryo" may have educational value for readers of the Adopting Release and Final Guidelines.
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> Further to this centrally important point about defining the class of human embryos that can give rise to stem cells that can, in turn, be used in research eligible for NIH funding, we note the scholarship of Louis M. Guenin, Lecturer on Ethics in Science in the Department of Microbiology and Molecular

[2] 45 CFR 202(c): "Fetus means the product of conception from implantation until delivery." NAS in the Glossary of its Guidelines for hESC research (rev. 2008) states: "Embryo—An animal in the early stages of growth and differentiation that are characterized by cleavage, laying down of fundamental tissues, and the formation of primitive organs and organ systems; especially the developing human individual from the time of implantation to the end of the eighth week after conception, after which stage it becomes known as a fetus."

> Genetics at Harvard Medical School. In his seminal work The Morality of Embryo Use, Guenin presents a compelling argument for the morality of using embryos that are donated to medicine, subject to a prohibition by their respective donors of intrauterine transfer. Guenin uses the term "epidosembryo" for such an embryo.[3] Guenin illustrates that the combination of the prohibition of intrauterine transfer with the scientific fact that development of the embryo requires implantation results in the epidosembryo having no embryonic development potential, thus forming the basis for the morality of using the epidosembryo for medical research. We note that while the 2000 NIH Final Guidelines contained a prohibition of intrauterine transfer of embryos, the Draft Guidelines does not. We also note that there may be other areas of life science research in addition to medicine where an epidosembryo approach to substantiating the morality of using hESCs/iPSCs has relevance – one such example being the use of hESCs for determining the toxicity of substances in the field of environmental health assessments.[4]

Each of the three paragraphs in the above excerpt illustrates a point that GPI believes has continuing relevance to the Guidelines.

First Paragraph in the Excerpt from the 2009 GPI Letter

The first paragraph in the above excerpt calls upon NIH to provide a glossary for the terms that are used in the Guidelines. In hindsight, GPI could have made a much simpler proposal to NIH: add a sentence that incorporates by reference into the Guidelines the NIH Stem Cell Glossary or, in the alternative, a one sentence cross reference that states that terms not otherwise defined in the Guidelines have the meanings given to them in the NIH Stem Cell Glossary. Revisiting the question of definitions in the Proposing Release provides an opportunity for NIH to consider this simple and often used technique of drafting regulatory and other legal documents.

If NIH were to take this proposed step, it would need to expend some further effort in sharpening some of the definitions in the NIH Stem Cell Glossary. For example, as we note above, the NIH Stem Cell Glossary defines hESC as "[a] type of pluripotent stem cell derived from the inner cell mass (ICM) of the blastocyst." Since this definition is inconsistent with the proposed revision of the definition of hESCs presented in the Proposing Release, it would need to be revised whether or not NIH adds a one-sentence incorporation by reference of the NIH Stem Cell Glossary into the Guidelines or an equally short cross reference from the Guidelines to the Glossary.

Second Paragraph in the Excerpt from the 2009 GPI Letter

The second paragraph in the excerpt from the 2009 GPI Letter points out that the U.S. National Academy of Sciences in its guidelines for research with hESCs and induced pluripotent

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[3] Guenin, Louis M. The Morality of Embryo Use (2008), p. 27. ("After the Greek epidosis, for an Athenian's beneficence to the common weal, I define the following: Epidosembryo. An epidosembryo is a human embryo as to which the following obtain: (a) The embryo was created outside the human body, and (b) the progenitors who contributed the gametes or other cells from which to form the embryo have donated the embryo, as of or after its creation, on the condition, set forth in written instructions accepted by the recipient, that (i) the recipient shall use the embryo solely in medical research or therapy, and (ii) never may the embryo or any totipotent cell taken from the embryo be transferred into a woman or into an artificial uterus.")

[4] See, e.g., CIRM. *Stem Cells in Predictive Toxicology*. CIRM Workshop Report July 7-8, 2008. ("Chapter 4. Stem Cells And Predictive Toxicology In Environmental Health Assessment.")

stem cells (first published in 2005 and amended in 2008) defines "embryonic stem (ES) cells" as "primitive (undifferentiated) cells derived from the early embryo that have the potential to become a wide variety of specialized cell types." The appearance of the word "early" in this NAS-crafted definition should remind us of the manner in which NIH used this word in the draft of the guidelines it adopted in 2000 for human stem cell research. NIH deleted the word "early" from the final version of the guidelines it promulgated in 2000 (and subsequently withdrew in the wake of the August 9, 2001 change in U.S. presidential policy regarding research with hESCs), but in doing so explained in the preamble to the final version that the word "early" serves to "make it clear that NIH funding of research using hPSCs derived in the private sector from human embryos can involve only embryos that have not reached the stage at which the mesoderm is formed." [5]

This reference in the preamble to the NIH 2000 guidelines to "embryos that have not reached the stage at which the mesoderm is formed" serves as a backdrop against which to consider the phrase "up to and including the blastocyst stage" currently proposed by NIH for the definition of hESCs. We appreciate that as we continue to fill the voids in our collective understanding of the continuum encompassed by embryogenesis,[6] phrases like "having not reached the stage at which the mesoderm is formed" may be ambiguous and thus not helpful for purposes of precision and interpretability. Therefore, it is better to use the phrase "up to and including the blastocyst stage" for purposes of defining hESCs in the Guidelines. But, like the term hESC, the term blastocyst as it is proposed for use in this revised definition also requires interpretable precision and clarity.

The NIH Stem Cell Glossary already defines the term blastocyst.[7] And we do not intend here to comment on the sufficiency of this definition for purposes of revising the definition of hESCs. But assuming that this definition is sufficiently precise and clear for use in the proposed revision of the definition of the term hESCs, we believe that in the Guidelines NIH should follow the definition of hESCs with a simple statement that indicates that the term blastocyst as so used in the definition of hESCs has the same meaning as that in the NIH Stem Cell Glossary. If the NIH Stem Cell Glossary is incorporated by reference into the Guidelines or otherwise cross referenced globally in the manner we described above, this suggested insertion would not be necessary.

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[5] NIH. "Guidelines for Research Using Human Pluripotent Stem Cells." August 25, 2000/Federal Register, Vol. 65, No. 166, p. 51975. [Corrected November 21, 2000] [Withdrawn by Federal Register, Vol. 66, No. 220, p. 57107] (NIH states in the Preamble: "Respondents suggested dropping the word 'early' throughout the document or more clearly defining 'early.' The word 'early' in reference to human embryos has been deleted; the Guidelines make it clear that NIH funding of research using hPSCs derived in the private sector from human embryos can involve only embryos that have not reached the stage at which the mesoderm is formed.")

[6] *See, e.g.*, Rossant J, Tam PP. *Blastocyst lineage formation, early embryonic asymmetries and axis patterning in the mouse*. Development. 2009 Mar;136(5):701-13. ("Despite recent advances, the exact mechanisms that link cell polarity, cell position, the effects of the local micro-environment, signaling activity and cell fate in blastocyst formation remain to be determined.")

[7] The NIH Stem Cell Glossary defines "blastocyst" as a "preimplantation embryo of about 150 cells produced by cell division following fertilization. The blastocyst is a sphere made up of an outer layer of cells (the trophoblast), a fluid-filled cavity (the blastocoel), and a cluster of cells on the interior (the inner cell mass)."

We also note here the impact of the proposed revision of the term "hESCs" on the phrase "early stage" in the definition of hESCs. The proposed revision sets forth an explicit upper bound on the developmental stage of the class of embryos from which derived hESCs are eligible for federal funding under the Guidelines. Setting forth explicitly this upper bound renders unnecessary and confusing the use of the phrase "early stage" in the definition of hESCs for purposes of the Guidelines (and similarly renders moot the GPI excerpted comment presented above).

Turning now to the period of time preceding the formation of the blastocyst, we know that the development of totipotent cells precedes the development of the blastocyst and that these totipotent cells differentiate into not only "pluripotent embryonic stem cells…of the blastocyst, [but] other progenitor cells…that self-renew in culture [and] can also be derived from the blastocyst, such as trophoblast stem (TS) cells…"[8] Since 1998, we have known that these other stem cells into which totipotent cells differentiate have vital scientific and medical relevance.[9] Relative to the literature on pluripotency, there seems to be a paucity in the scientific literature on totipotency.[10] This should help underscore NIH's reason for revising the definition of hESCs for purposes of the Guidelines: to assure the eligibility for federal research funding of stem cells derived from embryos that do not reach blastocyst stage. It should be clear that the totipotent cells in embryos that do not reach blastocyst stage as well as the stem cells in the blastocyst that are not derived from the inner cell mass need to be better understood, particularly in view of what we know of their scientific and medical relevance. Moreover, from the limited literature that does exist on totipotency we at least know that the markers that differentiate between totipotency and pluripotency may be worthy of further study.[11]

The above discussion underscores the need to understand the role of more than just pluripotent cells in the blastocyst. Accordingly, the definition of hESCs should be based on stem cells, not pluripotent stem cells, from embryos that have not developed beyond the blastocyst stage, as that stage is currently understood and subject to refinement as embryology and its canons continue to evolve.

Third Paragraph of Excerpt from 2009 GPI Letter

GPI believes that the epidosembryo concept proposed by Lou Guenin continues to be the most systematically thoughtful, well-defined, and ethically sound approach to addressing the morality of embryo use for scientific and medical purposes. Application of this concept can

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[8] Rossant and Tam, Note 6, ***supra***.

[9] Tanaka S. et al. *Promotion of trophoblast stem cell proliferation by FGF4*. Science. 1998 Dec 11;282(5396):2072-5. ("The trophoblast cell lineage is essential for the survival of the mammalian embryo in utero. This lineage ... is restricted to form the fetal portion of the placenta.")

[10] In a search of the PubMed database undertaken on March 23, 2010 with the string {totipotent[Title] AND "stem cells"[MeSH Terms]}, 24 articles were retrieved, compared to 835 articles retrieved for the search string {pluripotent[Title] AND "stem cells"[MeSH Terms]}, and 7,075 articles retrieved by the search string {embryonic[Title] AND "stem cells"[MeSH Terms]}.

[11] *See, e.g.*, Cauffman G, et al. *Markers that define stemness in ESC are unable to identify the totipotent cells in human preimplantation embryos*. Human Reproduction. 2009 Jan;24(1):63-70. Epub 2008 Sep 29.

provide a consistent mechanism for implementing inclusion of totipotent, pluripotent, and other stem cells in the definition of hESCs.

We also believe that the epidosembryo concept can serve as a compass to NIH's Working Group of the Advisory Committee to the Director in carrying out its responsibilities under Sections II.B.2 and II.C of the Guidelines. These two sections provide the steps for the Working Group to follow for embryo donations meeting certain chronological or geographical parameters specified in those two sections.[12]

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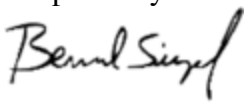
GPI appreciates the opportunity to have presented the above comments relating to the proposed change in the definition of hESCs in the Proposing Release and would be pleased to answer any questions or comments that NIH may address to us.

Respectfully submitted,



Bernard Siegel
Executive Director
Genetics Policy Institute

[12] Section II.B of the Guidelines covers embryo donations in the United States prior to July 7, 2009 (the effective date of the Guidelines); and Section II.C covers embryo donations outside the United States both prior to and on or after July 7, 2009.